

University of Rhode Island

DigitalCommons@URI

---

Open Access Master's Theses

---

1999

## Determination and Characterization of Water, Oil and Surfactant Induced Variations in Isotropic Domains of Plurololeique CC 497 / Labrasol System

Anchalee Ain-Ai

*University of Rhode Island*

Follow this and additional works at: <https://digitalcommons.uri.edu/theses>

---

### Recommended Citation

Ain-Ai, Anchalee, "Determination and Characterization of Water, Oil and Surfactant Induced Variations in Isotropic Domains of Plurololeique CC 497 / Labrasol System" (1999). *Open Access Master's Theses*. Paper 964.

<https://digitalcommons.uri.edu/theses/964>

This Thesis is brought to you for free and open access by DigitalCommons@URI. It has been accepted for inclusion in Open Access Master's Theses by an authorized administrator of DigitalCommons@URI. For more information, please contact [digitalcommons@etal.uri.edu](mailto:digitalcommons@etal.uri.edu).

DETERMINATION AND CHARACTERIZATION OF WATER, OIL, AND  
SURFACTANT INDUCED VARIATIONS IN ISOTROPIC DOMAINS OF  
PLUROL OLEIQUE CC 497 / LABRASOL SYSTEM

BY

ANCHALEE AIN-AI

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
MASTER OF SCIENCE

IN

PHARMACEUTICAL SCIENCES

UNIVERSITY OF RHODE ISLAND

1999

MASTER OF SCIENCE THESIS

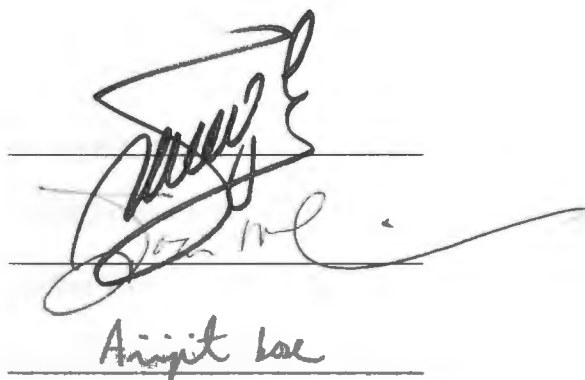
OF

ANCHALEE AIN-AI

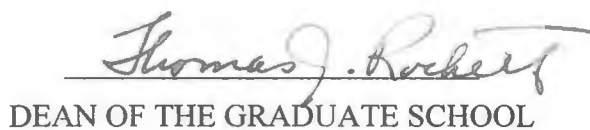
APPROVED:

Thesis Committee

Major Professor



A handwritten signature, likely of the Major Professor, is written over two horizontal lines. Below the signature, the name "Aungit Lee" is printed in a cursive font, also over a horizontal line.



A handwritten signature, likely of the Dean of the Graduate School, is written over a horizontal line. Below the signature, the name "Thomas J. Roberts" is printed in a cursive font, also over a horizontal line.

DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND

1999

## ABSTRACT

Although microemulsions have been widely investigated and applied in various industries, existing raw materials limit applications of microemulsions in pharmaceuticals and cosmetics. This work attempts to clarify the formation of isotropic systems when Labrasol/Plurololeique CC 497 combinations were used as the surfactant/cosurfactant combinations known as  $K_m = 0/1, 1/4, 2/3, 1/1, 3/2, 4/1, 1/0$ . The  $K_m = 1/1$  and  $3/2$  combinations provided widest isotropic areas that included maximum of 66.67% w/w of mineral oil, 48.73% w/w of soybean oil and 48.82% w/w of water for  $K_m = 1/1$ , whereas for  $K_m = 3/2$  provided maximum of 54.54 %w/w of mineral oil, 28.85% w/w of soybean oil and 54.55% w/w of water. Therefore  $K_m = 1/1$ (HLB 10) and  $3/2$  (HLB 10.8) combinations were further studied to determine their response to variables such as effect of oil polarity, effect of hydrocarbon versus triglycerides, temperature and ionic strength and pH of the aqueous phase.

The hydrocarbon (mineral oil) provided a wide isotropic area compared to a triglyceride (soybean oil). The effect of the mineral oil was investigated by adding a C18 fatty acid having no double bond (oleic acid), two double bonds (linoleic acid), and three unsaturated chains (linolenic acid). They have not produced a significant difference in the composition of isotropic areas. However at 10% concentration, they all reduced the isotropic domains significantly. Linolenic acid, which is the most polar one, is being the most effective.

The increasing concentration of Labrasol increased thermo-stability of the systems. At  $K_m = 3/2$  combination, the systems was also able to tolerate NaCl and  $\text{CaCl}_2$  up to ionic strength of 1. They can be used within pH 1.5 to 7.4 to various oil and water solubilizing capacities.

## ACKNOWLEDGEMENTS

First of all, I would like to express my sincere thanks to my advisor Prof. M. S. Kislalioglu for her support, patient and suggestions during the entire span of this research.

I also wish to express my gratitude to my thesis committee members: Dean J. Lausier, Dr. A. Bose, and Dr. L. Martin for their valuable suggestions and useful comments during the review of this thesis

Thanks to my dear friends whom I met at work and school: Fiona, Neha, Nepa, O.J., Thomas, Patti, Sameer, Sunny, John, Kelvin, and my brother for their valuable labor toward this thesis and for cheering me up during this difficult time in my life. Thanks to International office, department secretaries: Kathy and Mary. Their tremendous help during my study is truly appreciated.

I am grateful to my supervisor at Merck & Co.: Dr. K.C Thompson for her invaluable encouragement, mentorship, and kindness.

Finally I would like to extend my gratitude to my family in Thailand for their financial support throughout my study and for their love to this daughter. Without their help it would have been impossible to achieve the completion of this thesis.

## PREFACE

I selected to write this thesis following the standard manuscript format of the Graduate manual at the University of Rhode Island. In which the effect of oils, surfactant ratios, ionic strengths, fatty acids, pH and temperatures on isotropic domains in three phase diagrams were reported. It has been subdivided into seven sections.

Section 1 and 2 consist of a general introduction of the topic and the objectives of my investigation. Section 3 is the experimental techniques. The results obtained are discussed in section 4. The entire work is concluded in the section 5. Section 6 is the references supporting the entire thesis. The last section consists of bibliography.

## TABLE OF CONTENTS

	page
<b>1.0 INTRODUCTION</b>	
1.1 Definition and Distinctions Between Macroemulsions and Microemulsions	1
1.2 The Role of Surfactant and Cosurfactant	2
1.3 Determination of Microemulsions by Three Phase Diagrams	10
1.4 Characterization of Microemulsions	13
1.4.1 Particle size analysis	13
1.4.1.1 direct electron microscope imaging	14
1.4.1.2 light scattering methods	15
1.4.1.2.1 dynamic light scattering	15
1.4.2 Electrical conductivity	17
1.5 Effect of Components on Overall Characteristics	19
1.5.1 Effect of the oil phase	19
1.5.2 Effect of the aqueous phase	21
1.5.3 Effect of the the temperature	24
1.6 Application of Microemulsions	26
1.6.1 Pharmaceutical and cosmetic applications	26
1.6.2 Other fields	29



<b>2.0 OBJECTIVES</b>	<b>31</b>
<b>3.0 EXPERIMENTAL</b>	<b>33</b>
3.1 Materials	33
3.1.1 Nature and properties of Plurololeique CC 497 and Labrasol	34
3.1.2 Nature and properties of mineral oil, soybean oil and superpurified soybean oil	35
3.1.3 Nature and properties of oleic acid, linoleic acid and linolenic acid	37
3.2 Instruments	37
3.3 Methology	38
3.3.1 Construction of the three phase diagram	38
3.3.2 Characterization of isotropic domains	40
3.3.2.1 light scattering	40
3.3.2.2 conductivity	40
<b>4.0 RESULTS AND DISCUSSION</b>	<b>42</b>
4.1 Effect of Surfactant/Cosurfactant Ratios	42
4.2 Effect of Oil Type and Polarity	56
4.2.1 Influence of unsaturation of C 18 fatty acids	58

4.3 Effect of Temperatures	62
4.4 Effect of Aqueous Phases	68
5.0 SUMMARY AND CONCLUSIONS	77
6.0 REFERENCES	79
7.0 BIBLIOGRAPHY	86

## LIST OF TABLES

	page
1. List of Material used	33
2. List of Instruments used	38

## LIST OF FIGURES

	page
<b>1.2.1.</b> Insertion of oil molecules between hydrocarbon surfactant chain	4
<b>1.2.2.</b> Effect of surfactant/cosurfactant ratio in the phase transitions in microemulsions containing equal volumes of oil and water	9
<b>1.3.1.</b> Construction of a three phase diagram	12
<b>4.1.1.</b> Effect of Labrasol/Plurololeique CC 497 ratios on the isotropic domains of light mineral oil at 20°C.	43
<b>4.1.1.D.</b> Phase diagram of mineral oil microemulsion at surfactant/cosurfactant ratio ( $K_m$ ) of 1/1 at 20°C	44
<b>4.1.2.</b> Particle size and conductivity changes of the isotropic phases at increasing water content of the isotropic phases obtained at $K_m = 1/1$ of Labrasol/Plurololeique CC 497 and mineral oil combinations	45
<b>4.1.1E.</b> Phase diagram of mineral oil microemulsion at surfactant/cosurfactant ( $K_m$ ) = 3/2 at 20°C	47
<b>4.1.3.</b> Particle size and conductivity changes of the isotropic phases at increasing water content of the isotropic phases obtained at $K_m = 3/2$ of Labrasol/Plurololeique CC 497 and mineral oil combinations	49
<b>4.1.4.</b> Effect of Labrasol/Plurololeique CC 497 ratios on isotropic domains of soybean oil at 20°C	50
<b>4.1.4.D.</b> Phase diagram of soybean oil microemulsion at surfactant/cosurfactant ( $K_m$ ) = 1/1 at 20°C	52
<b>4.1.5.</b> Particle size and conductivity changes of the isotropic phases at increasing water content of the isotropic phases obtained at $K_m = 3/2$ of Labrasol/Plurololeique CC 497 and soybean oil combinations	53
<b>4.1.4.E.</b> Phase diagram of soybean oil microemulsion at surfactant/cosurfactant ( $K_m$ ) = 3/2 at 20°C	54
<b>4.1.6.</b> Particle size and conductivity changes of the isotropic phases at increasing water content of the isotropic phases obtained at $K_m = 3/2$ of Labrasol/Plurololeique CC 497 and soybean oil combinations	55

## LIST OF FIGURES (Cont'd)

	page
<b>4.2.1.</b> The isotropic domains obtained using Labrasol/Plurololeique CC 497 3/2 mixture, light mineral oil, soybean oil and water at $T = 25^{\circ}\text{C}$	57
<b>4.2.2.</b> Effect of superpurification of soybean oil on the isotropic domains of Labrasol/Plurololeique CC 497 ratio 3/2 mixture ( $K_m = 3/2$ ) isotropic phases at $T = 25^{\circ}\text{C}$	59
<b>4.2.3.</b> Effect of fatty acids (oleic acid, linoleic acid, linolenic acid) on the isotropic domains of mineral oil at Labrasol/Plurololeique CC 497 ratio 3/2, $T = 25^{\circ}\text{C}$	61
<b>4.3.1.</b> Effect of the various temperatures on the isotropic domains of mineral oil at Labrasol/Plurololeique CC 497 ratio 1/1	63
<b>4.3.2.</b> Effect of the various temperatures on the isotropic domains of soybean oil at Labrasol/Plurololeique CC 497 ratio 1/1	64
<b>4.3.3.</b> Effect of the various temperatures on isotropic domains of mineral oil at Labrasol/Plurololeique CC 497 ratio 3/2	66
<b>4.3.4.</b> Effect of the various temperatures on the isotropic domains of soybean oil at Labrasol/Plurololeique CC 497 ratio 3/2	67
<b>4.4.1.</b> Effect of electrolyte types and concentrations on isotropic domains of mineral oil at Labrasol/Plurololeique CC 497 ratio 1/1, $T = 25^{\circ}\text{C}$	69
<b>4.4.2.</b> Effect of electrolyte types and concentrations on isotropic domains of soybean oil at Labrasol/Plurololeique CC 497 ratio 1/1, $T = 25^{\circ}\text{C}$	70
<b>4.4.3.</b> Effect of electrolyte types and concentrations on isotropic domains of mineral oil at Labrasol/Plurololeique CC 497 ratio 3/2, $T = 25^{\circ}\text{C}$	71
<b>4.4.4.</b> Effect of electrolyte types and concentrations on isotropic domains of soybean oil at Labrasol/Plurololeique CC 497 ratio 3/2, $T = 3/2$ , $T = 25^{\circ}\text{C}$	72
<b>4.4.5.</b> Effect of the aqueous phase on the isotropic domains of mineral oil at Labrasol/Plurololeique CC 497 ratio 3/2, $T = 25^{\circ}\text{C}$	75
<b>4.4.6.</b> Effect of the aqueous phase on the isotropic domains of soybean oil at Labrasol/Plurololeique CC 497 ratio 3/2, $T = 25^{\circ}\text{C}$	76

## **1.0 INTRODUCTION**

### **1.1 Definition and Distinctions Between Macroemulsions and Microemulsions**

The term microemulsion defines spontaneously formed stable disperse systems in which the average particle size of the internal droplets range from 10-200 nm.<sup>1</sup> As a result, a microemulsion appears as a transparent single - phase liquid.<sup>2</sup>

Microemulsions usually consist of three to five components: an oil phase, an aqueous phase, and a primary surfactant. In many cases, they contain a secondary surfactant (cosurfactant), and sometimes one or more electrolytes. These isotropic systems are usually more difficult to formulate than ordinary emulsions because their formation is a highly specific process that involves spontaneous interactions of the constituents. The type of the association structures that can form with these components at a particular temperature, depends not only on the chemical nature of each component but also on their relative concentrations.<sup>3</sup>

While macroemulsions are ordinarily opaque or white in color, the microemulsions are transparent or translucent (opalescent) in a state which reflects their much smaller particle size (generally less than 1/4 the wavelength of light).<sup>4</sup> This is one of the properties that attracted the attention of researchers from various applied sciences.

Recent studies have emphasized the stability and ease of formation of microemulsions rather than their transparency. Macroemulsions, by contrast, are generally thermodynamically unstable. Moreover macroemulsion manufacturing generally requires an application of a form of external energy, while microemulsions tend to form spontaneously.

Because they are isotropic and thermodynamically stable systems with large interfacial areas, microemulsions may be superior to macroemulsions for solubilization of poorly soluble drugs.<sup>5,6,7,8</sup> Thus, microemulsions may be considered as viable alternatives to macroemulsions for both cosmetic and pharmaceutical applications.

## **1.2 The Role of Surfactant and Cosurfactant**

Surfactants are the chemicals which contain both hydrophilic and hydrophobic groups. The hydrophobic group is not miscible with water molecules and when a small concentration of surfactant is added to water, the hydrophobic end will immediately rise to the surface in order to reduce its free surface energy.<sup>3</sup> This structure is called a monolayer. If the concentration of surfactant is increased and there is not enough room for all of the hydrophobic ends to escape from the water, a bilayer may form. However, if the concentration of surfactant is large enough, the bilayer may form which will not be stable. At this point, the excess surfactant molecules form micelles. In a micelle, a group of hydrophilic heads shield their hydrophobic tails from the water. Therefore, the micelle that form in the water consists of a hydrophilic shell and a hydrophobic core.

The concentration of the surfactant at which the micelle formed is called the critical micelle concentration (CMC).<sup>9,10,11</sup>

Surfactant films at oil and water interfaces determine many properties of the microemulsions such as the phases, particle size and stability. The hydrophilic surfactant films tend to curve towards oil and form o/w emulsions because they will interact with water molecules more than oil molecules. The surfactants, which can reduce the interfacial tension excessively and interact with oil and water molecules spontaneously may form microemulsions. The spontaneous curvature ( $H_0$ ) of the interfacial film depends on the hydrophilic nature of the surfactant. Spontaneous curvature ( $H_0$ ) is the most important parameter to understand the structure and stability of the microemulsions which depend on the nature of the surfactant, oil, and water phases.

As shown in Fig. 1, the oil molecules penetrate to some extent between the hydrocarbon tails of the surfactant molecules. The more extensive the penetration, the deeper the curvature is toward the water side causing a reduction in  $H_0$ . When the curvature is toward the oil,  $H_0$  is positive, when it is towards water it is negative.<sup>12</sup>

When the spontaneous curvature of the surfactant film,  $H_0$ , is greater than zero,  $H_0 > 0$ , o/w is expected, and if  $H_0 < 0$ , w/o can be formed. A stable microemulsion can be formed when the spontaneous curvature ( $H_0$ ) of the film is close to the inverse radius ( $R^{-1}$ ) of the droplet. If the spontaneous curvature is much higher than the inverse radius ( $H_0 \gg R^{-1}$ ), the surfactant film tends to move toward a lower free energy state by decreasing



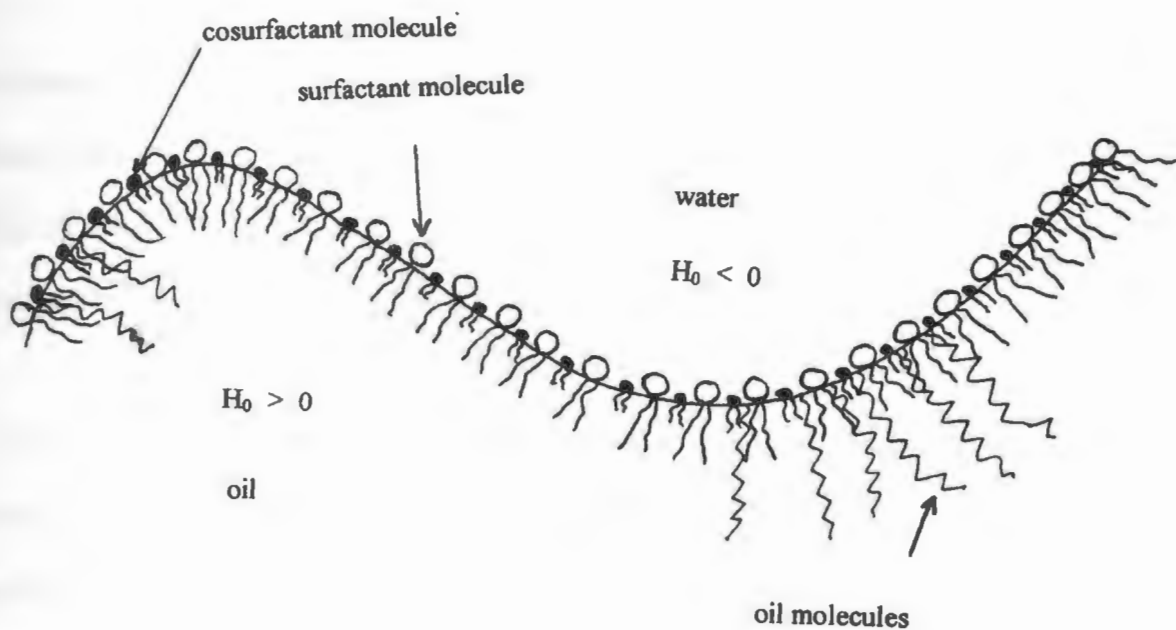


Fig.1.2.1: Insertion of oil molecules between hydrocarbon surfactant chain. The greater extent of penetration causes the surfactant film to curve toward water. (From Ref. 12 with modification)

the droplet size and expelling the emulsified oil (or water for negative  $H_0$ ) to the bulk phase. When  $H_0 \ll R^{-1}$ , the film forms larger nonspherical aggregates. Finally when  $H_0 \sim 0$ , there is no preference for a o/w and w/o structure; therefore a bicontinuous phase can be formed.<sup>12</sup>

In formation of microemulsions, spontaneous adsorption is an important factor. Not every surfactant can be spontaneously located at the interface and reduce the interfacial tension near or below zero values. In order to do that, the surfactant should have comparable oil and water affinities.

For example, n-alkyl polyoxyethylene ethers are able to form microemulsions by themselves. They are single-chained surfactants. Some of the double chain surfactants such as di-2-ethyl-hexyl-sulphosuccinate (AOT) and di-octadecylammonium bromide (DDAB) can form microemulsions due to their favorable geometry and their ability to reduce interfacial tension. However, theories related to microemulsification are complicated. Some authors like Shinoda et al.<sup>13</sup> suggested the role of the critical packing parameter (CPP) and related geometry of the surfactant in forming microemulsions. Authors like Malcolmson and Lawrence<sup>14</sup> working with a variety of POE ether surfactants demonstrated that, the procedure involved in microemulsion formation is more complex and depends on the number and size of the hydrophobic tails and the hydrophilic ethylene oxide of the surfactant.

Cosurfactants are small surface-active molecules. They tend to interact with the surfactant and modify its interfacial properties. They are mostly short-chain alcohols ( $C_1$ - $C_{10}$ ). Cosurfactants which can form microemulsion are short chain alcohols, such as propanol, butanol and pentanol<sup>20</sup> and other substances, such as alcanoic acids (n-hexanoic acid, n-pentanoic acid), amines (n-alkylamines, cyclohexylamine) and polyethylene glycol alkyl ethers (diethylene-glycol-monobutyl-ether, diethylene-glycol-monopentyl-ether).<sup>15,16</sup>

There are several molecules suitable for use as cosurfactants. Their effects in microemulsion formation may be variable. Those with medium chains, like pentanol and hexanol formed microemulsions by reducing the relative affinity of the nonionics and water molecules. On the other hand, the lower chained alcohols were found to have an opposite effect.<sup>17</sup> Since the use of such alcohols may not be suitable for use in pharmaceuticals and cosmetics, the molecules that can be used as cosurfactants in these industries are highly limited.

Cosurfactants, due to their small molecular weight and surface activity, can easily mix with the surfactant, the water, and the oil phases. They reduce the interfacial tension further and adjust the viscosity of the interfacial film. In the presence of the cosurfactant, the interfacial film becomes more fluid and hence can easily and spontaneously curve to form very small droplets<sup>11</sup>. In the presence of the cosurfactant, the HLB of the interfacial film is also changed. This is so because many authors reported the formation

of the microemulsion at an optimum surfactant and cosurfactant combination having an HLB which is able to solubilize both oil and water phases.<sup>18</sup>

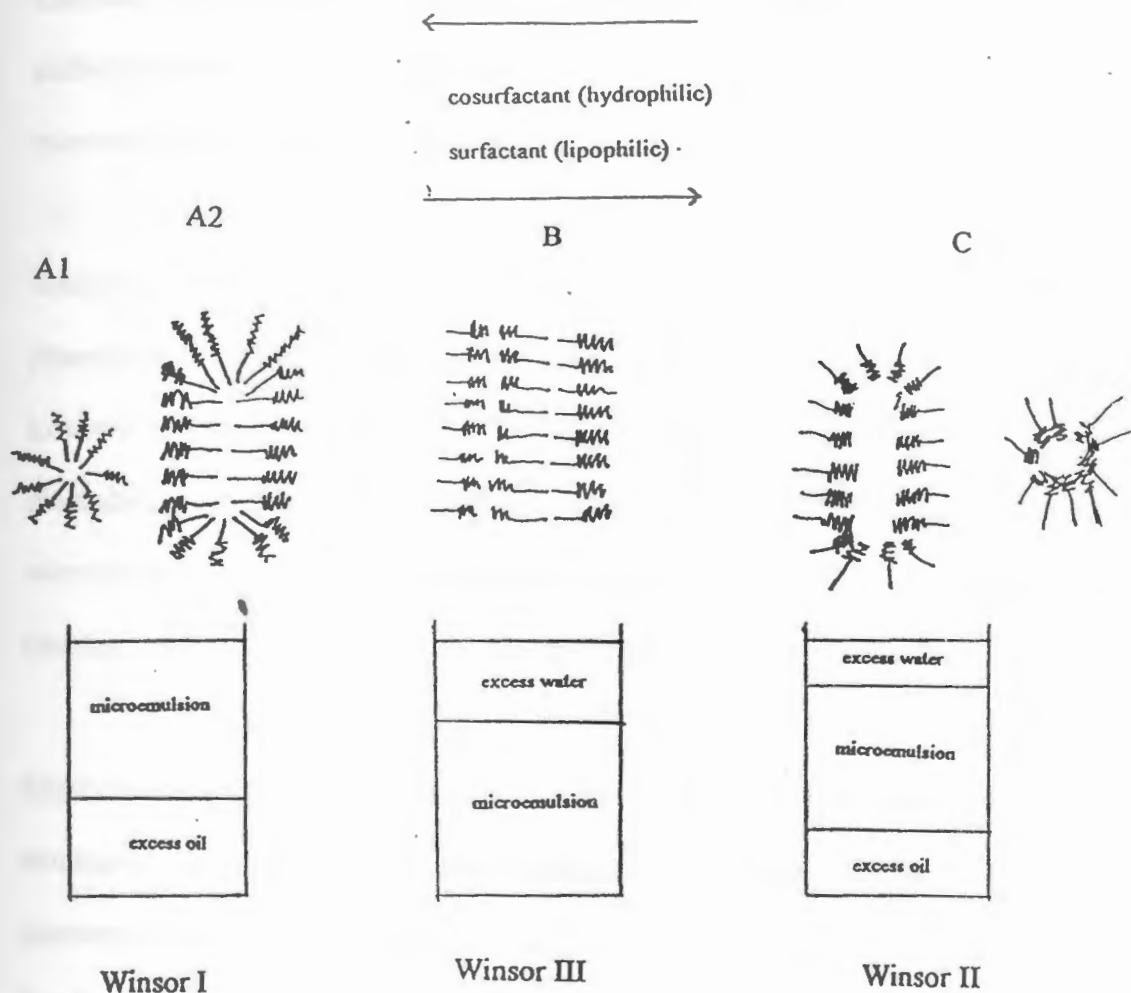
An optimum hydrophilic-lipophilic balance (HLB)<sup>13</sup> and critical packing parameter (CPP)<sup>15</sup> are readily accepted concepts for the microemulsion formation, although not every surfactant may not fit to the prediction. Malcolmson and Lawrence found that CPP of a surfactant fails to predict the production of microemulsions<sup>19</sup>.

As Lawrence<sup>11</sup> reported, most surfactants are not able to form microemulsions of oil and water without a cosurfactant. Shinoda and Friberg<sup>20</sup> have suggested that in order to enhance the solubilizing power, the size of the hydrophilic and lipophilic groups of the surfactant and cosurfactant should be increased. This may be achieved by varying surfactant/cosurfactant ratios until an optimum HLB number at the maximum solubilization of oil and water could be obtained. Therefore it is obvious that the extent of isotropic domains depend upon the HLB<sup>21</sup>.

Every isotropic domain found in oil-surfactant/cosurfactant and water mixtures is not a microemulsion. They may be true solution of surfactant and/or cosurfactant, micelles in oil or in the aqueous phases, microemulsions, or liquid crystals. While changing the surfactant/cosurfactant ratio, the HLB of the surfactant mixture is also changed and this change in HLB influences the structure and distribution of the oil-surfactant-water phases.

Winsor system was used to classify the structural changes that took place in the surfactant-oil-water mixtures. Phase transitions involved is shown in Fig. 2. When increasing amounts of a lipophilic surfactant is added to a cosurfactant dissolved in equal amounts of oil/water mixture, a range of self assembly structures form due to changes occur in the HLB mixture. These were named as phase I, II, and III by Winsor, Fig.2. Normally, cosurfactant and surfactant micelles change from normal to swollen micelles and form oil in water microemulsions (Winsor Type I, o/w). Increasing surfactant concentration reverts it to middle phase (Winsor Type III) and finally to reverse micelles where a water in oil emulsion forms (Winsor Type II). Figure 2 shows various Winsor types for a water-oil system containing equal volumes of water and oil. At A, an excess cosurfactant is added to the surfactant. The surfactant mixture is very hydrophilic and the surfactant/cosurfactant forms micelle with a portion of the oil phase partitioned into the micellar core ( $A_1$ ). When hydrophilicity decreases, a swollen micelle ( $A_2$ ) forms. This is also referred to as Winsor I. At further decreasing hydrophilicity, a continuous phase occurs (Winsor III) (B). In the presence of a small amount of cosurfactant the continuous phase is the oil phase. Winsor II systems are swollen micelles in oil and in the absence of the cosurfactant, reverse micelles form.<sup>10,13,18</sup>

Kahlweit et.al.<sup>22</sup> studied the microemulsification of mineral oils by using a biological surfactant, lecithin. It was found that soybean lecithin can considerably enhance the efficiency of nonionic n-alkyl polyglycol ethers in microemulsifying alkanes.



Various Winsor Phases

Fig.1.2.2: Effect of surfactant/cosurfactant ratio in the phase transitions in microemulsions containing equal volumes of oil and water. (From Ref. 10, 13, 16 with modification)

Literature reviews demonstrated that Polysorbate 80, Labrasol and lecithin are the surfactants that are likely to form microemulsions.<sup>5,23,24</sup> Attwood et al<sup>25</sup> have prepared microemulsions by using Polysorbate 60 as a surfactant and sorbitol as a cosurfactant.

Selection of the components, especially the surfactants to form a microemulsion for pharmaceutical applications should be carried out by carefully considering their toxicity, irritancy, and sensitizing properties<sup>26</sup>. Although ionic surfactants such as dioctadecylammonium bromide (DDAB), n-alkyl-polyoxyethene ethers can be used successfully in many industrial applications, they have the limitation to be used in pharmaceutical and cosmetic products due to their toxicity.<sup>11</sup>

Microemulsions are thermodynamically stable and can display a range of self-assembly structures. The most commonly known types are o/w and w/o systems. Typically, the microemulsion drops are nearly spherical and have a small polydispersity. The radius of a droplet ( $R$ ) is normally one to four times of the thickness ( $l_o$ ) of the surfactant film. The excess surfactant available remains in the continuous phase.

### **1.3 Determination of Microemulsions by Three Phase Diagrams**

A microemulsion is assumed to be the mixture of four components; water phase, oil phase, and surfactant/cosurfactant mixture. The ratios of those four components that form a microemulsion can be best shown on the three phase diagram.<sup>27,28,29</sup> Three phase diagrams are ideal for initial screening of microemulsions formulations and normally are constructed from data gathered either by titration or by the preparation

of a large number of samples of different compositions.<sup>30</sup> A phase diagram is used to determine appropriate concentrations of the components that will form a microemulsion. Overall the titration techniques are very useful to determine the gross phase boundaries in such systems.<sup>20,31</sup>

An advantage of the titration method is that it can be used to study a large number of compositions relatively quickly, however, the rate of the study might obscure the results because the transfer of one phase to another may reach equilibrium in a longer time-frame like 24 hours. The systems for each combination should be observed for 24 hours to detect possible phase transformations.

Fig.3 shows how to construct a phase diagram by varying the oil and surfactant ratio in oil-surfactant/cosurfactant (S/Cos) mixtures by titrating them with the aqueous phase. A surfactant/cosurfactant mixture that was dissolved in oil was titrated with water. The water titration process was shown with arrows drawn towards water corner.  $A_1$ ,  $A_2$ , and  $A_3$  represent fixed ratios of the S/Cos that were chosen by the formulator. If the isotropic phases that were prepared in this manner, have not been monitored for sufficient time intervals (usually 24 hours) to determine whether they have reached equilibrium, they tend to provide false information. Once an appropriate amount of oil, water, and a surfactant/cosurfactant mixture is determined by titration and plotted on a phase diagram, it can be produced spontaneously simply by blending oil, water, surfactant, and cosurfactant with mild agitation.

The order in which the different components are added to the mixture does not affect the formulation of the microemulsion in a properly selected system.



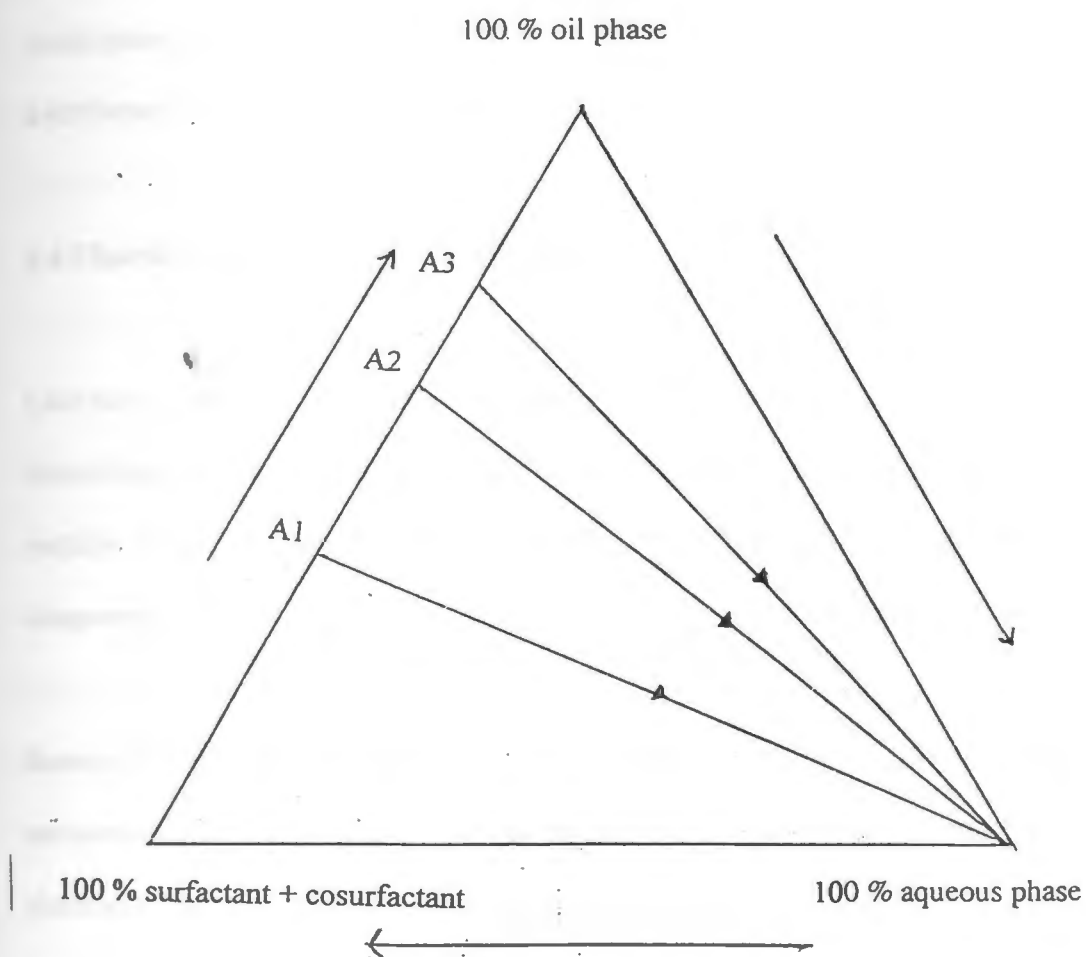


Fig.1.3.1: Construction of a three phase diagram (Usually fixed ratios of surfactant/cosurfactant is used. They are mixed with the oil phase and titrated with the aqueous phase).

A w/o microemulsion is normally easier to produce than an o/w microemulsion. Ro Samo et al.<sup>32</sup> suggested a simple three-step procedure for preparing an o/w microemulsion, using titration method. First, a primary surfactant that is only slightly soluble in the oil phase is dissolved in the oil phase to yield a w/o microemulsion. This mixture is added to the water phase and stirred then titrated with a cosurfactant that is more soluble in water than a surfactant to produce a clear o/w microemulsion.

#### **1.4 Characterization of Microemulsions**

Like macroemulsions, microemulsions may be of o/w or w/o type. The cubical bicontinuous phase is also accepted as a microemulsion. Their mean particle size and particle size distribution may differ, depending on the nature and the quantity of their components.

Some of the physical measurements that are useful in characterizing microemulsions are conductivity, light scattering, optical birefringence, ultra-centrifugation, rheology, electron microscopy, nuclear magnetic resonance, and photon correlation spectroscopy.<sup>33,34-37</sup>

##### **1.4.1 Particle size analysis**

The transparency of microemulsions arises from their small droplet size. Therefore it is very important to carry out particle size analysis since any variation in droplet size may

indicate changes in the stability of microemulsions<sup>38</sup> and solubilization properties of the particles.<sup>39</sup>

Many techniques have been employed in the size analysis of microemulsions such as direct electron microscope imaging, light scattering method, small angle neutron scattering, and photon correlation spectroscopy. Because of the limitations of each technique, it is preferable to employ a combination of these techniques for any microemulsion size analysis.<sup>30,37,40</sup>

#### 1.4.1.1 direct electron microscope imaging

Thin portions of the microemulsion specimen are directly investigated in a frozen hydrated state by using a liquid cryogen in the transmission electron microscope. The development of glass-forming microemulsions that neither break down nor crystallized during cooling process provided a way for direct studies of the microemulsion structures.<sup>41</sup> The fact that neither phase crystallized on cooling below 0°C meant that possible modifications of microemulsion structure by crystal growth were avoided.<sup>37</sup> This technique was applied by Green<sup>42</sup> who used a glass-forming microemulsion containing oil (ethyl cyclohexane), glycerol/water mixtures as the dispersed phase, and diiododecyl dimethyl ammonium bromide as the surfactant to make up an w/o microemulsion. Direct electron microscope imaging of the microemulsion indicated droplets of 20 nm diameter.

Evan<sup>12</sup> has shown the structure of a bicontinuous microemulsion containing 7 wt% C<sub>12</sub>E<sub>5</sub>, water, and octane. The sample was prepared by freeze-fracture and subsequent deposition of a tantalum-tungsten coating.

#### 1.4.1.2 light scattering methods

Scattering methods are the small angle x-ray scattering (SAXS),<sup>36</sup> small angle neutron scattering (SANS),<sup>37</sup> and static and dynamic light scattering.<sup>43</sup> They all have a lower size limit of approximately 2 nm and an upper limit of approximately 100nm for SANS and SAXS and a few microns for light scattering. In general terms, all light scattering instruments contain the same components: a light source (usually a laser), a spectrometer (containing the optical components for defining the scattering angle volume), a detector (usually a photomultiplier), a signal analyzer such as a spectrum analyzer or a correlator, and a computer software for analysis are standard for dynamic measurements.<sup>44</sup>

Gorski et al.<sup>38</sup> have determined the particle size of an o/w microemulsion system as around 30-100°A by using SANS and static light scattering. The microemulsion system was consisted of tetradecyltrimethylammonium bromide (TTABr) as a surfactant, 1-hexanol as a cosurfactant, decane as an oil phase, and water.

##### 1.4.1.2.1 dynamic light scattering (DLS)

The dynamic light scattering (DLS) was used to measure the changes occurred in the particle size of the emulsions studied in this thesis. This method accurately measures the particle size and allows evaluation of the interactions that happen between different structures that may be present in the microemulsion systems.<sup>31</sup>

Colloidal particles immersed in fluid and in the solution form are able to scatter a beam of the light. The scattering pattern depends on the particle sizes and on the wavelength of the light. Lord Rayleigh was introduced the equation for the scattered intensity,  $I$ , where the particles are much smaller than the wavelength of the light:

$$I/I_0 = 9\pi^2 (N_1^2 - N_0^2)^2 / 2\lambda^4 R^2 (N_1^2 + 2N_0^2)^2 V^2 N_p (1 + \cos^2 \theta) \dots (1)$$

where  $I_0$  is the initial intensity of unpolarized light,  $\theta$  is the angle between the incident beam and scattered beam,  $N_p$  is the isolated particles per unit volume,  $V$  is the particle volume,  $N_1$ ,  $N_0$  are the refractive indices of particles and medium respectively.  $R$  is the distance where the intensity  $I$  is measured from the particles and  $\lambda$  is the wavelength of visible light.

In this equation, the term  $1$  and  $\cos^2 \theta$  refer to the vertical and horizontally polarized component of the scattered light.<sup>40</sup>

The use of lasers has a great advantage to study the light scattering by providing coherent, monochromatic, intense and narrow incident beams, coupled with sensitive stable photon-detection apparatus and rapid data analysis by computer.<sup>40</sup>

The temporal variation of the intensity is measured and is represented usually through the intensity auto-correlation function. The diffusion coefficients of the particle, particle sizes, and size distribution can be deduced from such measurements. The variation of the intensity with time, contains information on the random motion of the

particles and can be used to measure the diffusion coefficient of the particles. The measured diffusion coefficient can then be used to determine the size of the particle.<sup>40,45,46</sup>

Sjoblom and Friberg<sup>41</sup> have investigated phase transitions in the microemulsion systems containing water, pentanol, and potassium oleate by varying water content but with constant alcohol/soap ratios. They found that at low water concentrations no rise of light scattering was observed when Rayleigh radii ( $R$ ) was plotted against volume fraction.

Electron microscopy of carbon replicas from freeze-fractured samples used in association with particle size measurements demonstrated the presence of molecular dispersions and absence of inverse micelles at low water content. At higher water concentrations, a slight change from a negative to positive slope of the curve was observed at approximately 15 % (w/w) for all series and the first association occurred when the water molecules per surfactant exceeded eight and the inversed micelles were formed in increasing water content of the mixture.

Bond et al.<sup>45</sup> have investigated the structural transitions in w/o microemulsions in the three-phase diagram by following the particle size changes with light scattering. They concluded that the system had low conductivity within w/o region. In the upper region of w/o which was closer to surfactant corner, the surfactant was in excess relative to the amount of aqueous phase to be solubilized, rendering the systems to hydrated surfactant aggregates.

#### 1.4.2 Electrical conductivity

Measurement of electrical conductivity with a conductometer was proposed many years ago as a way of differentiating between O/W and W/O emulsions. Because, a water-continuous phase would be expected to show a much higher conductivity than an oil-continuous phase <sup>45</sup>.

Usacheva et al.<sup>1</sup> have studied the conductivity of single phase microemulsions containing Brij 97, n-butanol, dodecane, and water at 20°C. From the conductivity data, they determined the water concentration that caused transition of the emulsion to water in oil type. For example, Zourab and Miller<sup>47</sup> reported conductivities reduced from 148  $\mu\Omega^{-1}$  to 14  $\mu\Omega^{-1}$  when the system reverted from o/w to w/o. During the phase inversion of Brij-97, n-butanol, and dodecane systems, the conductivity jumped from  $10^{-6}$  to  $10^{-4}\Omega^{-1}$ .

Baker et al<sup>46</sup> investigated structural changes occurring in the system by following the conductivity-viscosity changes and particle size measurements. These explained the nature of the phase changes took place in sodium-alkyl-benzene-sulphonate-hexanol-water combination.

Lagues and Sautery<sup>48</sup> reported that conductivity jumps changes from  $10^{-7}$  s.cm<sup>-1</sup> for w/o phase to  $10^{-2}$  s.cm<sup>-1</sup> obtained with o/w emulsion. In the study carried out by Joubran et al<sup>31</sup> with soybean oil, polyoxyethylene(40), sorbitol hexaoleate, water ,and ethanol, conductivity values changing from 2 to 25  $\mu\Omega/\text{cm}$  were interpreted as the phase transformation of the system from w/o to o/w.

Although they are valuable, conductivity studies by themselves have limited value to determine the exact properties of the phases during transformation.

## 1.5 Effect of Components on Overall Microemulsion Characteristics

### 1.5.1 Effect of the oil phase

The need for the oil to associate with the interfacial film of the microemulsion means that the size of the oil molecules is important in determining whether a microemulsion is formed. As a rule, greater solubilization is achieved with oils with smaller molecular weight than the surfactants.<sup>49</sup> If the molecular weight of the oil is very large, no microemulsions form.<sup>11</sup> The penetration ability of oils to the o/w interface is important. The hydrocarbons with shorter chain-lengths e.g. C<sub>6</sub> can penetrate into the C<sub>12</sub> surfactant chains more readily than longer alkyl chains containing 12 hydrocarbons whereas the C<sub>14</sub> and C<sub>16</sub> chains do not penetrate to the interface at all. Thus the microemulsion cannot be formed.<sup>40</sup> The higher the penetration ability of the oil, the more curvature of the oil-water interface occurs and this phenomena can decrease the water droplet sizes of w/o microemulsions. For example they range from about 4 nm for hexane to about 10 nm for dodecane.<sup>40</sup>

Published data on the microemulsion technology emphasized the use of non-polar carbons such as decane<sup>49</sup>, heptane, hexadecane, and octadecane.<sup>14</sup> Studies that have been carried out with more polar triglycerides are rare.<sup>14,31</sup> Alander and Warenheim<sup>50</sup> have compared triglyceride behavior in the microemulsions with ordinary hydrocarbons such as mineral oil, decane, and heptane. They concluded that triglycerides, especially large triglycerides such as peanut oil, are considerably more difficult to be solubilized into microemulsions than the hydrocarbons.



The vegetable oils are triglycerides that are easily metabolized in the body therefore they can be used in the parenteral products. Recently by removing the active impurities such as polar components and oxidative impurities, those oils that were further purified were referred to as "superrefined oils". Such oils are known to improve the chemical stability of the drugs incorporated in them.<sup>51</sup> However, their effect on the dispersed systems and their stability have not been studied. Lobo and Kislalioglu<sup>52</sup> have demonstrated that the superpurification process has not influenced the stability of the multiple emulsions prepared with soybean oil (USP). Soybean oil contains triglycerides of oleic acid 26%, of linoleic acid 49%, of linolenic acid 11%, of saturated acids 14%<sup>53</sup>. They are polar oils that may not favor microemulsion formation.<sup>54</sup>

Effect of oils that have short alkyl chains and their interaction with the surfactant may favor microemulsion formation. On the other hand, it appears that the small oils can deeply penetrate the interfacial surfactant layer(s) and deleteriously modify the surfactant film which is one of the main sites of solubilization in the microemulsion.<sup>11</sup> If the microemulsion will be a drug delivery system, it may be advantageous to use oils having larger alkyl chains than that of the surfactants which are less likely to penetrate the surfactant film if the solubilization in microemulsions is important. Malcolmson et al.<sup>54</sup> have studied the level of solubilization of testosterone propionate into 2% o/w microemulsions stabilized by a nonionic surfactant (polyoxyethylene 10-oleyl ether, Brij 96). They found that the microemulsions, containing the larger molecular volume oil, caprylic/capric triglyceride, solubilized the hormone three times as much of those containing ethyl butyrate which have a small alkyl chain.

Polar oils do not favor formation of the microemulsions. However pharmaceutically acceptable oils tend to have 12 or more alkyl chains and semipolar in nature.<sup>11,14</sup> Thus it is important to investigate the effect of such oils on the microemulsion formation.

Aboofazeli et al.<sup>18</sup> have studied the effect of polarity of the oils in lecithin microemulsions. Six polar oils were examined including soybean oil, Miglyol 812, ethyl oleate, oleic acid, ethyl octanoate, and octanoic acid with increasing polarities in the given order. They found that the extent and composition of various solubilization regions were dependent largely on surfactant/cosurfactant ratios and the nature of the oil being incorporated. The larger isotropic domains occurred when the small oils were used. Overall, although the differences in phase behavior were observed, with different oils these differences were small, except when using the large triglycerides as oils. It appears that the size, rather than the polarity of oils investigated in this study is the main factor influencing phase behavior.

In contrast to triglycerides, mineral oils are the mixtures of saturated hydrocarbons. They are mainly used for oral and topical applications in the pharmaceutical industry and in the food and cosmetic industries. Light mineral oil is listed in the USP as a laxative. Mineral oils are excellent moisturizers and emollients and have been used in the formulas for baby oils, creams and lotions, bath oils, lipsticks and lip gloss, sunscreens, hair products and make-up bases and removers<sup>55</sup>. Therefore the effect of light mineral oil in the formation of microemulsions was further investigated in this study.

### 1.5.2 Effect of the aqueous phase

In the pharmaceutical or cosmetic microemulsions, the aqueous phase is usually the core that contains the drug matter or the active substances. The drug may be neutral in nature, an electrolyte, a weak acid or base. All of these have the tendency to modify the ionic character of water phase. Among the surfactants that can form microemulsions the ionic ones are found to be more sensitive to the presence of electrolytes than the nonionic ones<sup>59</sup>. An electrolyte can penetrate into the head group of surfactants causing dehydration and shrinking of the size of the head group thus causing instability.<sup>60</sup> Compared to ionic surfactants, the nonionic surfactants are less prone to the changes caused by the changing pH, or to the presences of electrolytes than the ionic surfactants, therefore they are preferred in the pharmaceutical and cosmetic delivery systems.

Kahlweit et. al.<sup>61</sup> indicated that the inorganic electrolytes can be divided into two groups, the first is the lyotropic salts such as NaCl and CaCl<sub>2</sub> which decrease the mutual solubility between water and surfactant. The second group comprised of the hydrotropic salts such as NaClO<sub>4</sub> and (C<sub>6</sub>H<sub>5</sub>)<sub>4</sub>PCl which increase the mutual solubility. When NaCl was introduced to H<sub>2</sub>O-C<sub>12</sub>E<sub>6</sub> and H<sub>2</sub>O-C<sub>12</sub>E<sub>5</sub> systems, the surfactant became more lipophilic. In contrast, the hydrotropic NaClO<sub>4</sub> increased the hydrophilicity of the surfactant.

The effect of electrolyte concentration on the solubilization capacity in nonionic microemulsions was investigated by Wiencek and Qutubuddin.<sup>62</sup> The oil studied were the normal C<sub>6</sub>-C<sub>16</sub> alkanes. Neodol 91-2.5 (Shell) and DNP-8 (Emery) were used as the nonionic surfactants. The aqueous phase consisted of NaOH and KSCN solutions of varying concentrations. It was found that depending upon the type of electrolyte used, the

solubilization capacity of the nonionic microemulsions was increased or decreased.

Generally, typical electrolytes such as NaCl and CaCl<sub>2</sub> decrease the solubilization capacity in increasing concentrations. However KSCN showed the opposite behavior.

Gan-Zuo et al.<sup>63</sup> have demonstrated that the ionic interaction between the surfactant/cosurfactant (AOT/butanol) and ionic species present in aqueous phase influence the microemulsification regions by NaCl. The added salt reduced the critical micelle concentration (CMC) of the surfactant system and the increased ionic strength reduced the electrostatic repulsion (compression of the electrical double layer) between the droplets and micelles, resulting in instabilities. With increasing ionic strength, some part of o/w structures are shrunk because the effective polar part was less bulky than the hydrophobic part and the o/w structures are not favored when nonionic surfactant becomes more hydrophobic.<sup>6</sup> Similarly the solubility of ether-type surfactants in water rests on the formation of hydrogen bonds between water and the ether oxygen atom. When these hydrogen bonds are broken, or at least destabilized by heat and/or the presence of some electrolytes, the solubility of the surfactant is materially reduced causing a transition of microemulsion from o/w to w/o systems<sup>14</sup>.

In pharmaceutical and cosmetic emulsions, besides the surfactant and cosurfactant type and properties, the pH of the aqueous phase is also highly important. Garcia-Celma et al.<sup>5</sup> have studied solubilization of antifungal agents using a microemulsion system containing a nonionic surfactant (POE (20) sorbitan monooleate), water and isopropyl palmitate, used as the oil phase. The selected drugs were clotrimazole, ciclopirox olamine and econazole nitrate ranging from slightly water soluble to practically insoluble.

Data obtained clearly showed that the model drugs could be successfully solubilized but the pH of the aqueous phase may effect the properties of microemulsion system and it may also influence the solubilization of weakly acidic or basic drug in the microemulsion.

De Vos et al.<sup>64</sup> investigated solubilization and stability of indomethacin, a weak acid, in a model transparent oil-water gel (TOW gel) as a function of pH of the water phase. The model TOW gel was composed of Cetiol<sup>®</sup>HE (polyoxyethylene (7) glyceryl monococoate) 15% w/w, an Eumulgin<sup>®</sup> B3 (polyoxyethylene (30) cetostearyl ether) 15% w/w, isopropyl palmitate 5% w/w, and demineralized water ad 100% w/w. Indomethacin, being a weakly acidic drug is poorly soluble in the acidic environments. Although its solubility was increased in the alkaline medium, high pH hydrolyzed the drug. Solubilizing indomethacin in the TOW gels increased the stability of the drug to 300 days at pH 5.6 and 7.8.

### 1.5.3 Effect of the temperature

Temperature-sensitivity is one of the commonly known features of the nonionic surfactants, and may result in the phase transformation. The nonionic surfactants are more temperature sensitive than the ionic ones because at high temperatures the hydrophilic ethylene-oxide groups become dehydrated rendering the molecule to lipophilic state. At a critical temperature, the surfactant solubility in water decreases and the surfactant solution becomes cloudy which is called Cloud Point. This temperature is referred as "Phase Inversion Temperature, PIT" by Shinoda<sup>56</sup>. The PIT can also be viewed as that point or

that temperature at which a surfactant that is an oil-in-water emulsifier becomes a water-in-oil emulsifier.

At this temperature, there is a delicate balance between the hydrophilic and lipophilic groups and the emulsion formed using such surfactants can be easily reverted from o/w to w/o phase by slight temperature variations around this temperature. The temperature sensitivity of a microemulsion prepared with the nonionic surfactants may be affected by the surfactant/cosurfactant ratio and the number of ethylene-oxide groups present in the surfactant.

Friberg et al<sup>57</sup> found that nonionic surfactants at their PIT, frequently formed microemulsions over a wide range of compositions. Generally as the temperature increased, the surfactant shifted its preferential solubility from water to oil and at temperatures below or above PIT, a higher amount of surfactant was needed to obtain the isotropic solutions.

Fletcher and Morris<sup>39</sup> have investigated a decane/water microemulsion stabilized by a C<sub>12</sub>E<sub>5</sub> nonionic surfactant. The microemulsions were formed over a narrow temperature range.

At low temperatures, photon correlation spectroscopy and turbidity measurements indicated that the droplets were spherical and interacted only weakly. With increasing temperatures, the size of the particles arising from either clustering or coalescence of the droplets caused turbidity. They recommended the use of turbidimetric detection of the temperature-jump technique to investigate the kinetics of the clustering/growth process.

This concept finds utility in emulsification technology. Therefore it is important to formulate the microemulsions within the PIT range to optimize the conditions and maximize the solubilization of oil and water during the microemulsion preparation. The PIT of the final formulation can be changed as the result of the oil type and surfactant ratio.<sup>31,49</sup> The PIT appears to be a linear function of the HLB value of the surfactant. The higher the HLB value, the greater the PIT will become. When the ratio of the hydrophilic to lipophilic moieties in a surfactant molecule increases, a higher temperature is needed to dehydrate it to the point where its structure is balanced.<sup>58</sup>

In practice, the regular storage temperature of dosage forms and cosmetics may change between -8°C - 35°C. The ambient temperature is 25°C, the surface of the skin is 32°C, and the temperature of the lower epidermis is 37°C. A microemulsion should remain stable within the manufacturing, storage and skin temperature ranges. Joubran et al.<sup>31</sup> demonstrated that Tween 40 and sorbital/hexaoleate/ethanol stabilized soybean emulsions were temperature sensitive between 20-30°C. Similarly many microemulsions may be unstable within narrow temperature ranges if they are stabilized by nonionic surfactants. If increasing temperature causes a phase inversion of a o/w to w/o emulsion or causes a microemulsion to revert to a macroemulsion within narrow temperature ranges, the related system is not suitable for use as a drug delivery system.

## **1.6 Application of Microemulsions**

### **1.6.1 Pharmaceutical and cosmetic applications**

Over the last ten years microemulsions have attracted a considerable amount of interest as potential drug delivery systems. Part of this interest arise from their clarity , ease of

preparation, stability, ability to be filtered, low viscosity and no pain upon injection since the droplets are small in a range of nanometers.<sup>11</sup> These properties combined with their potential for incorporating a range of drugs have made microemulsions suitable as drug delivery systems. Our interest in the pharmaceutical applications of microemulsions due to their easy preparation, high stability, solubilizing properties and ability to improve the stability of drugs.<sup>11</sup>

In **pharmaceutics**, microemulsion can be used as parenteral, oral, intranasal, rectal and topical drug delivery systems.<sup>26</sup> Topical application of microemulsions demonstrated increased concentration of drugs at the sites of delivery. For example, lecithin w/o microemulsions increased transport of methotrexate through intact hairless mouse skin significantly.<sup>65</sup> However, their use other than “topical” is limited due to high surfactant/cosurfactant concentrations needed for preparation.<sup>66</sup>

Microemulsions were used to enhance the oral bioavailability of poorly absorbed, hydrophilic or hydrophobic compounds such as cyclosporin, piroxicam, clonazepam, clofazimine<sup>23,67,68</sup> and drug solubilization of intravenous delivery of lipophilic and amphiphatic compounds.<sup>69</sup>

Constatinides<sup>6</sup> using self-emulsifying microemulsions containing polyglycolized glycerides reported that they can improve dissolution and oral absorption of indomethacin as compared to an aqueous suspension of the drug. Hammad and Muller<sup>8</sup> have investigated the solubility and stability of clonazepam in the bile salt/ soya phosphatidylcholine-mixed micelles (BS/SPC-MM). By comparison with Pluronic F-68,



a sugar ether and bile salt (BS), the BS/SPC-MM proved to be superior in increasing the solubility of clonazepam. In addition, chemical stability of clonazepam increased six fold in the shelf-stability obtained in the presence of BS/SPC-MM.

Jayakrisnan et al.<sup>66</sup> have formulated a microemulsion with Brij 35 and Arlacel 186, hexadecane oil, and isopropanol used as the cosurfactant. The solubility of hydrocortizone in the microemulsions was comparable to that in isopropanol alone. However it was found to be six fold higher in the microemulsions containing six gram of the total surfactant, 10 ml of oil, and 3.5 ml of alcohol at a water-to-oil ratio 0.10.

To improve the *in-vitro* dissolution of indomethacin, a self-microemulsifying drug delivery system (SMEDDS) has been formulated by Farah et al.<sup>70</sup> Gelucire 44/14 (Saturated polyglycolysed glycerides, HLB 14) and Gelucire 48/09 (Saturated polyglycolysed glycerides, HLB 9) were used as a lipophilic phase. Labrafac CM 10 (Saturated C8-C10 polyglycolysed glycerides, HLB 10) was used as a surfactant while Lauroglycol (Propylene glycol laurate, HLB 4) and Transcutol (diethyl glycol monoethyl ether) were the cosurfactants. It was found that these mixtures has the ability to form a microemulsion when exposed to gastrointestinal fluids and thus greatly enhanced the bioavailability of indomethacin.

For cosmetic use, both oil or water continuous systems are suitable. Oil-in-water systems are used in toilet preparations, for example, solubilized bath oils and perfumes may be formulated as transparent o/w emulsions.<sup>71</sup> In addition, microemulsion vehicles can act as carriers for hair relaxers or permanent wave solutions or depilatories.<sup>72</sup>

They are suitable products for cleansing, treating hair, eyelashes or eyebrows. The patented products assigned to L'Oreal consist of nonionic surfactants or a mixture of nonionic and cationic surfactants. The oil is described to be consisted of an amphiphilic lipid phase whose weight ratio oil-to-lipid changes from 2 to 10. The oil droplets have an average size of less than 150 nm. The nonionic amphiphilic lipid are from the silicone surfactants and polyolesters such as esters of polyethyleneglycol, sorbitan, ethoxylated glycerol or polyglycerols.

For example, avocado oil and ethanol are mixed with polyethylene-glycol-isosterate and N-stearoyl-l-glutamate disodium salt then added into the water-glycerin mixture and the system was homogenized. The result gave o/w nanoemulsion whose droplet size was approximately 63 nm.<sup>73</sup>

Another example is an antiperspirant microemulsion in the spray form which was reported to contain 0.5-30% of an oil phase, 0.1-30% of an antiperspirant salt, 0.35-30% of a nonionic hydrophilic emulsifying agent (especially an alkyl polyglucoside) and 0-7.5% of a lipophilic emulsifying agent. The dispersed-oil phase in the finished composition had a particle size ranging from 10 to 100 nm.

### 1.6.2 Other fields

Microemulsion formulations are used in emulsified oils such as self-polishing products, floor waxes, and car washes, in recirculating cutting oils to reduce the temperature rise

and provide smooth operation in the milling machines. Cutting oils consisted of a mineral oil for lubrication, diethyleneglycol as the cosurfactant, a soap (petroleum sulphonate) as a corrosion inhibitor, and water as the coolant. Pine-oil microemulsions are used as wetting and dispersing agents in the detergents. They are also used in flavour emulsions which are stabilized by a nonionic surfactant. Pesticide emulsions containing chlordane, emulsion polymers used as surface polishes also available in the market.

There have been an considerable interest in using vegetable oil microemulsions as replacements for diesel fuels, however the oil alone results in fuel injection coking and lubricant contamination due to its high viscosity. Finally there is an increasing interest in the use of micellar systems for modifying and controlling chemical reaction systems. In polymerization processes, microemulsion have been used to encapsulate offensive products. Similar uses are reported in the acid-base equilibria, the hydrolysis of esters, the formation of metalloporphyrins, photochemical and electrochemical systems.<sup>40</sup>

## 2.0 OBJECTIVES

Working with polyglycolysed glycerides (Labrasol) and polyglyceryl oleate (Plurololeique CC 497), as the surfactant/cosurfactant mixtures, water, mineral oil and soybean oils, the objective of this study was to determine the isotropic regions on the three phase diagram and investigate the effects of temperature, pH, ionic strength and type of ions on the isotropic phase boundaries.

Such combinations may provide multiple uses in cosmetic and especially in pharmaceutical technology since they are easy to manufacture, remain stable during the shelf-life and maintain their original characteristics during administration.

The specific objectives of this study were as follows:

- a. To determine an optimum ratio of Polyglycolysed glycerides (Labrasol) to Polyglyceryl oleate (Plurololeique CC 497) that provides the largest isotropic domain with mineral oil, soybean USP, purified soybean oils, and water. Hence Labrasol is used as a surfactant and Plurololeique CC 497 is used as cosurfactant.
- b. To find out the effects of oil type (mineral oil, soybean USP and purified soybean oils) on these microemulsion domains.

c. To mechanistically determine the effect of unsaturation of the  $C_{18}$  fatty acids on the microemulsion regions. This will be carried out by adding oleic, linoleic and linolenic acids to mineral oil and investigating their effect on the microemulsion domains.

d. To find out the effects of ion type, ionic strength and pH on soybean and mineral oil microemulsions by adjusting the ionic strength of the aqueous phase to 0.06 and 1 ionic strengths with NaCl and  $CaCl_2$ . (The pH effect will be studied by adjusting the pH of the aqueous phase to 1.5 with hydrochloric acid buffer (USP), to pH 5.5 with distilled water and to pH 7.4 with phosphate buffer, USP).

e. To investigate the effect of temperature on the soybean and mineral oil microemulsions by exposing the systems studied to 20, 25, 32 and 37 °C temperatures.

### 3.0 EXPERIMENTAL

#### 3.1 Materials

The materials used in this study are listed in Table 3.1.1. The properties of surfactants, oils and fatty acids are given in more detail in 3.1.1, 3.1.2 and 3.1.3.

Table 3.1.1: Materials used

Materials	Lot No.	Manufactures
Plurololeique cc 497 (polyglyceryl oleate)	15646	Gattefosse (Westwood, N.J.)
Labrasol (polyglycolysed glycerides)	15549	Gattefosse (Westwood, N.J.)
Mineral oil	R37817628	Ruger Carnation (Irvington, N.J.)
Soybean oil, USP	14	Croda Mill Hall, PA
Superpurified soybean oil	SB3-223NP	Croda Mill Hall, PA
Oleic acid	26,804-6	Aldrich Chem Co (Milw., WI)
Linoleic acid	L-1376	Sigma (St. Louis, MO)
Linolenic acid	L-2376	Sigma (St. Louis, MO)
Sodium chloride	N/A	Ruger Chemical (Irvington, N.J.)
Calcium chloride	48F-0010	Sigma (St. Louis, MO)
Potassium phosphate (monobasic)	923899	Fisher Chemical (Fair lawn, N.J.)
Sodium hydroxide	897895	Fisher Chemical (Fair Lawn, N.J.)
Potassium chloride	974251	Fisher Chemical (Fair Lawn, N.J.)
Hydrochloric acid	905754	Fisher Chemical (Fair Lawn, N.J.)

### 3.1.1. Nature and Properties of Labrasol and Plurololeique CC 497

#### 3.1.1.1 Labrasol

Labrasol is a mixture of saturated polyglycolized C<sub>8</sub>-C<sub>10</sub> glycerides. It is an oily liquid with HLB of 14. It is very soluble in ethanol, chloroform, methylene chloride and water, and is insoluble in mineral oil.

It is a powerful water soluble solubilizer for the pharmaceutical and cosmetic preparations. It can also be used as an enhancer in oral liquid and capsule formulations. It shows no oral irritation, but has a slight irritating effect on the ocular membrane.

It is a useful surfactant for microemulsion formulations. Its critical micelle concentration (CMC) is 0.3% (w/w). It is used in combination with a hydrophobic co-emulsifier to stabilize the microemulsion systems<sup>24,74</sup>.

#### 3.1.1.2 Plurololeique CC 497

Plurololeique CC 497 is polyglyceryl oleate. It is a viscous liquid with HLB 6. It does not have critical micelle concentration because it cannot form micelles by itself. It is freely soluble in chloroform, methylene chloride, vegetable oils and mineral oils. It is soluble in ethanol and dispersible in water.

It is a high viscosity emulsifier and used as a major constituent of the microemulsions that can be administered orally or topically. It is also a good suspending and stiffening agent.

It can improve the dissolution of most drugs and vitamins which can be filled into capsules. It is a GRAS substance and therefore can be safely used in the pharmaceutical and cosmetic applications. It has been found to be the best co-emulsifier with Labrasol to form microemulsions<sup>24,74</sup>.

### 3.1.2 Nature and properties of mineral, soybean and superpurified soybean oils

#### 3.1.2.1 mineral oil

Mineral oils are high boiling petroleum fractions obtained from crude oil distribution that are purified and refined by treatment with sulfuric acid. Mineral oil is a mixture of saturated hydrocarbons consisting of straight-chain (alkanes), branched (isoalkanes) and ring structures (cycloalkanes).<sup>75</sup> Light mineral oil is mainly composed of isoalkanes and cycloalkanes with carbon numbers ranging from 15 to 25.<sup>55</sup>

Light mineral oil USP NF XVII has HLB of 10-11<sup>76</sup>, specific gravity of 0.810-0.875, viscosity less than 33.5 cSt at 40°C. It is soluble in chloroform, ether, and hydrocarbons; sparingly soluble in 95% ethanol; and practically insoluble in water.<sup>77</sup>

Light mineral oil is a colorless, clear and odorless liquid, insoluble in alcohol or water.

Mineral oils are mainly used in topical drug and cosmetic preparations. They are excellent moisturizers and emollients as well as suitable lipophilic bases in which active ingredients can be delivered. Formulas for baby oils, creams and lotions, bath oils, lipsticks and lip



gloss, sunscreen and hair products contain Mineral Oil, USP or Light Mineral Oil NF, in the concentrations ranging from less than 1% to approximately 99%.<sup>55</sup>

It is likely that in most people, acute chronic exposure to mineral hydrocarbons either orally or topically may produce carcinogenesis. Because of the toxicity of mineral oil, vegetable oils been receiving increased attention.<sup>78</sup>

### 3.1.2.2 soybean and superpurified soybean oils

Soybean oil is obtained from soybeans by solvent extraction using petroleum hydrocarbons or, to a lesser extent, by expression using continuous screw press operations<sup>79</sup>.

The soybean oil that we used consists of triglycerides of oleic acid (26%), linoleic acid (49%), of linolenic acid (11%), saturated acids (14%), phospholipids, lecithin (1.5% to 4%). The remaining 0.8% consists of stigmasterol, sitosterols, and tocopherols. It has the following specifications: iodine value: 127-138, saponification value: 189-195, and acid value: 0.3-3.0.<sup>53</sup> According to the manufacturer<sup>80</sup>, superpurified soybean oil was composed of palmitic acid (7-14%), stearic acid (1-6%), oleic acid (19-30%), linoleic acid (44-62%), and linolenic acid (4-11%). Its saponification value is 180-200 and the iodine value is 120-141. It contains less than 1.0% unsaponifiable matter and has a specific gravity of 0.916-0.922 at 25°C. It contains no antioxidant and has a refractive index of 1.465-1.475. The refining process removes polar impurities such as peroxides, ketones, sterols, free fatty acid, tocopherols, and mono-diglycerides from the oil.

Soybean oil is a pale-yellow to brownish-yellow oil with slightly characteristic odor and taste. Due to the natural source, better skin compatibility compared to synthetic materials is expected. However, triglycerides are relatively problematic to use in cosmetic emulsions.

In addition to the difficulty of emulsification, the triglycerides can also become rancid due to the presence of unsaturated fatty acids. Consequently, antioxidants usually have to be added to the soybean oil. Superpurified soybean oil contains no polar impurities and hence enhances the stability of dosage forms. It is colorless, odorless and tasteless and is commonly used in parenteral formulations.<sup>51</sup>

### 3.1.3 Nature and properties of oleic, linoleic and linolenic acids

Oleic acid is found in all oils and fats. It has an open formula of  $\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$ . Linoleic acid is also among the regular constituents of vegetable oils. It is found in natural glycerides in small to substantial proportions. It has an open formula of  $\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$ . Linolenic acid is found in many natural glycerides in very small amounts and has an open formula of  $\text{CH}_3(\text{CH}_2\text{CH}=\text{CH})_3\text{CH}_2(\text{CH}_2)_6\text{COOH}$ .<sup>81</sup> The purities of oleic, linoleic and linolenic acids used in this study were ~99%, greater than 99% and ~99% respectively.<sup>82</sup>

## 3.2 Instruments

The instruments used in this thesis study are listed in Table 3.2.1. In addition to disposable scintillation vials (Kimble Glass, Inc., Vineland, N.J.), disposable culture tubes (Fisher Scientific, Pittsburgh, PA), and 0.05  $\mu$  filters were used.

### 3.3 Methodology

#### 3.3.1 Construction of three phase diagrams

First, appropriate amounts of Labrasol were mixed with Plurololeique cc 497 at a desired surfactant/cosurfactant ratio (Km) of = 0/1, 1/4, 2/3, 1/1, 3/2, 4/1, and 1/0 at 20°C. These mixtures were then mixed with the selected oil (light mineral oil or soybean oil) to provide oil/surfactant-cosurfactant ratios (R) of 1/9, 2/8, 3/7, 4/6, 5/5, 6/4, 7/3, 8/2, and 9/1. The mixtures were stirred until homogenous transparent mixtures were obtained. Solutions obtained were kept in a constant temperature water bath.

Table 3.2.1: List of Instruments

Instruments	Model No.	Manufacturer
Light scattering	BI-9000AT	Brookhaven instrument (Holtsville, N.Y)
Conductometer	13059052	EXTECH instruments (Taiwan)
Analytical Balance	1601 MP8	Sabron-Brinkman Instrument (Westbury, NY)
Mettler PT 200 balance	69547	Mettler instrument corp. (Princeton, N.J.)
Water bath	1063	Blue M Electric Company (Blue Island, IL)

The water phase was also kept at 20°C in a water bath and was added to the oil/surfactant mixture with a syringe, until a transparent phase was obtained. Water added was weighed and recorded after each addition and percent of each constituent in the mixture was calculated. The mixture was replaced in the water bath after thorough mixing and observed visually for the turbidity development. If it did not turn turbid, it was accepted as an isotropic phase. Only those mixtures which formed clear solutions were considered to be acceptable isotropic systems. Those were further analyzed by polarized microscopy to determine whether they contained any liquid crystal phases. Each titration was carried out three times.

The pH, ionic strength and temperature effects were studied by using surfactant/cosurfactant ratio of  $K_m = 3/2$  and  $1/1$ , which provided the largest isotropic areas on three phase diagrams. The pH effect was studied by adjusting the pH of the aqueous phase to 1.5 with hydrochloric acid buffer (USP), to 5.5 with distilled water and to 7.4 with phosphate buffer (USP). The ionic strengths were adjusted to 0.06 and 1 M with NaCl and CaCl<sub>2</sub>. The temperature effect was investigated at 20, 25, 32 and 37°C.

The effects of fatty acids were studied by adding adding 1% and 10% of oleic, linoleic and linolenic acids to light mineral oil respectively and investigating their effect on the microemulsion domains at 25 °C. The temperature effect was investigated at  $K_m = 3/2$ , which provided the widest isotropic areas on three phase diagrams. The effect of oil type (hydrocarbon versus. triglycerides) was also studied by using surfactant/cosurfactant ratio of  $K_m = 3/2$  at 25°C. The oils used were mineral oil, soybean oil, and superpurified soybean oil.

### 3.3.2 Characterization of Isotropic Domains

The main purpose of this study was to construct isotropic domains using light mineral oil and soybean oils. A few light scattering and conductivity results conducted, provided some information about the isotropic phases obtained at different compositions.

#### 3.3.2.1 light scattering

The measurements of scattered light intensity were made on a light scattering instrument, Model BI-9000 AT (Brookhaven Instruments, Holtsville, NY). The scattering intensities at 90° were recorded by a photomultiplier. Dust particles were removed from the solutions by passing it through a 0.45 µm Millex-HV filter. Microemulsion samples were prepared in test tubes (Fisher Scientific, Pittsburgh, PA), which were then tightly sealed by plastic caps and placed in a water bath at 20°C before the measurements. Three replicates were measured for the particle size determination. The particle sizes were directly obtained from the instrument's output.

#### 3.3.2.2 Conductivity

The conductivity measurements of the microemulsions were performed by using a conductance meter (Extech Instruments, Taiwan) at 20°C. The conductance was measured by immersing the positive and negative electrodes into scintillation vials (Kimble Glass Inc., Vineland, NJ) containing the isotropic phases. The conductance

values were measured in  $\text{ohm}^{-1} (\Omega^{-1})$  and the values obtained were used to plot conductivity relative to the aqueous phase concentration.

#### 4.1. EDC

The un-

equilibrium

in water

is always

of any

Fig. 4.1

surface

and by

from

compared

7.6) and

of

disturbance

Fig. 4.1

Effect of

conductivity

4.1.5 The

integrated

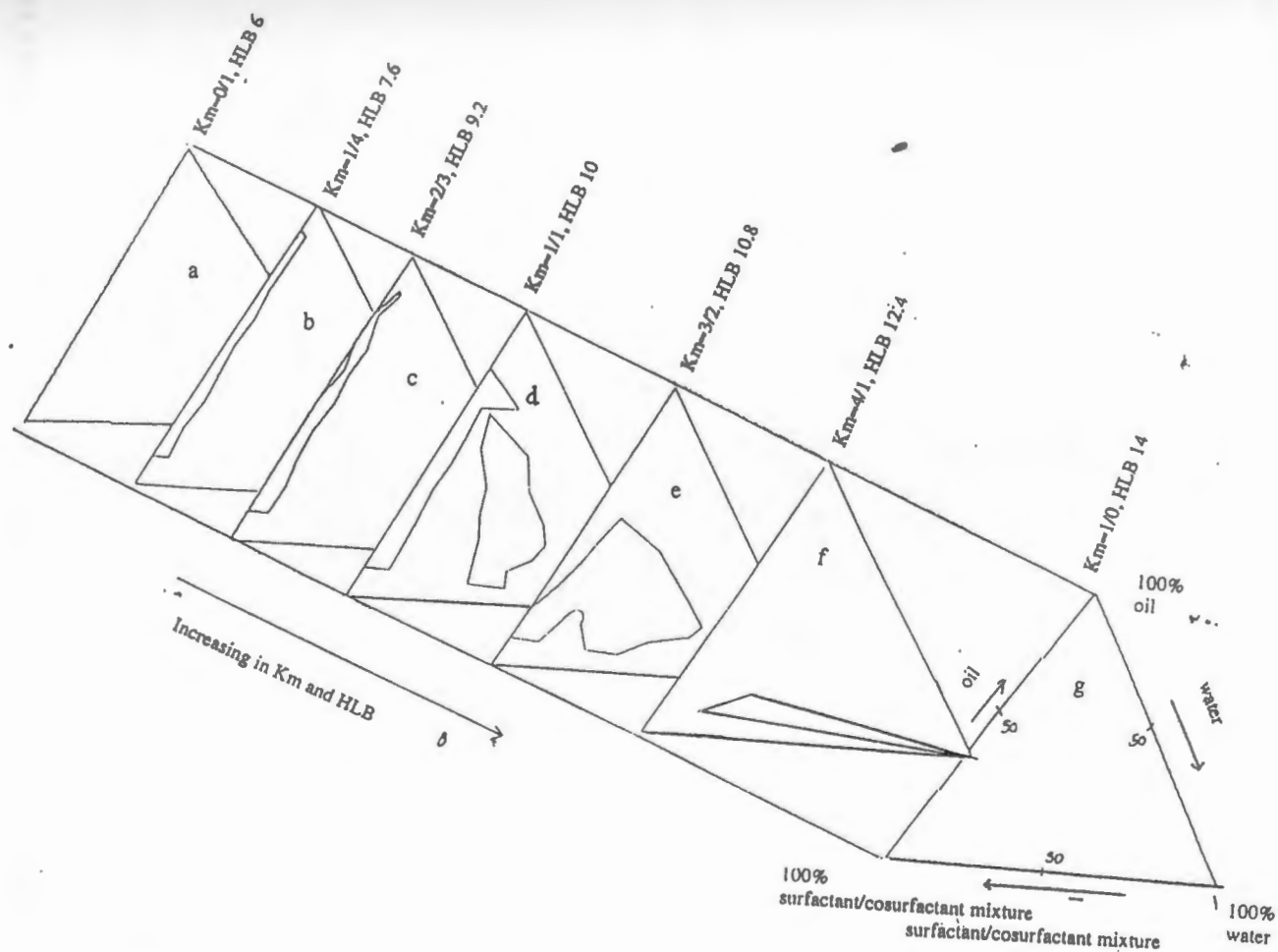
in

## 4. RESULTS AND DISCUSSION

### 4.1 Effect of Surfactant and Cosurfactant ratios

The nature and the characteristics of Plurololeique CC 497 and Labrasol are already mentioned in experimental section 3.1.1. Labrasol is insoluble in mineral oil and soluble in water, whereas Plurololeique CC 497 is freely soluble in mineral and vegetable oils but is dispersible in water. When combined together, their properties may facilitate formation of o/w and w/o microemulsions.

Fig. 4.1.1 demonstrates the isotropic phases obtained at different combinations of surfactant/cosurfactant (Labrasol/Plurololeique CC 497) ratios and mineral oil. When used by itself, neither Labrasol, Fig. 4.1.1(g), nor Plurololeique CC 497, Fig. 4.1.1(a), can form isotropic phases. When Labrasol is added into Plurololeique CC 497 in increasing concentrations, the system begins to demonstrate isotropic regions. At  $K_m = 1/4$  (HLB 7.6), isotropic phase is at the oil/surfactant corner, Fig. 4.1.1(b). The increased amounts of surfactant increase oil solubilization up to 87.04%. At  $K_m = 1/1$  (HLB 10), the mixture demonstrates two different isotropic regions, Fig. 4.1.1(d). To view these regions better, Fig. 4.1.1.d is enlarged as Fig. 4.1.1.D. Some information about the nature of the isotropic phase was determined by particle light-scattering and conductivity. The particle size and conductivity changes caused by increasing water content are demonstrated further in Fig. 4.1.2. Fig. 4.1.2 demonstrates the particle size and conductivity changes that occur in the isotropic region upon increasing the water content. At low water concentrations between 0-5.66%



4.1.1 Effect of Labrasol/Plurololeique CC 497 ratios on the isotropic domains of light mineral oil at 20°C. Km = 0/1: Plurololeique CC 497 (0%)/Labrasol (100%), Km= 1/0 Plurololeique CC 497 (100%)/Labrasol (0%)..



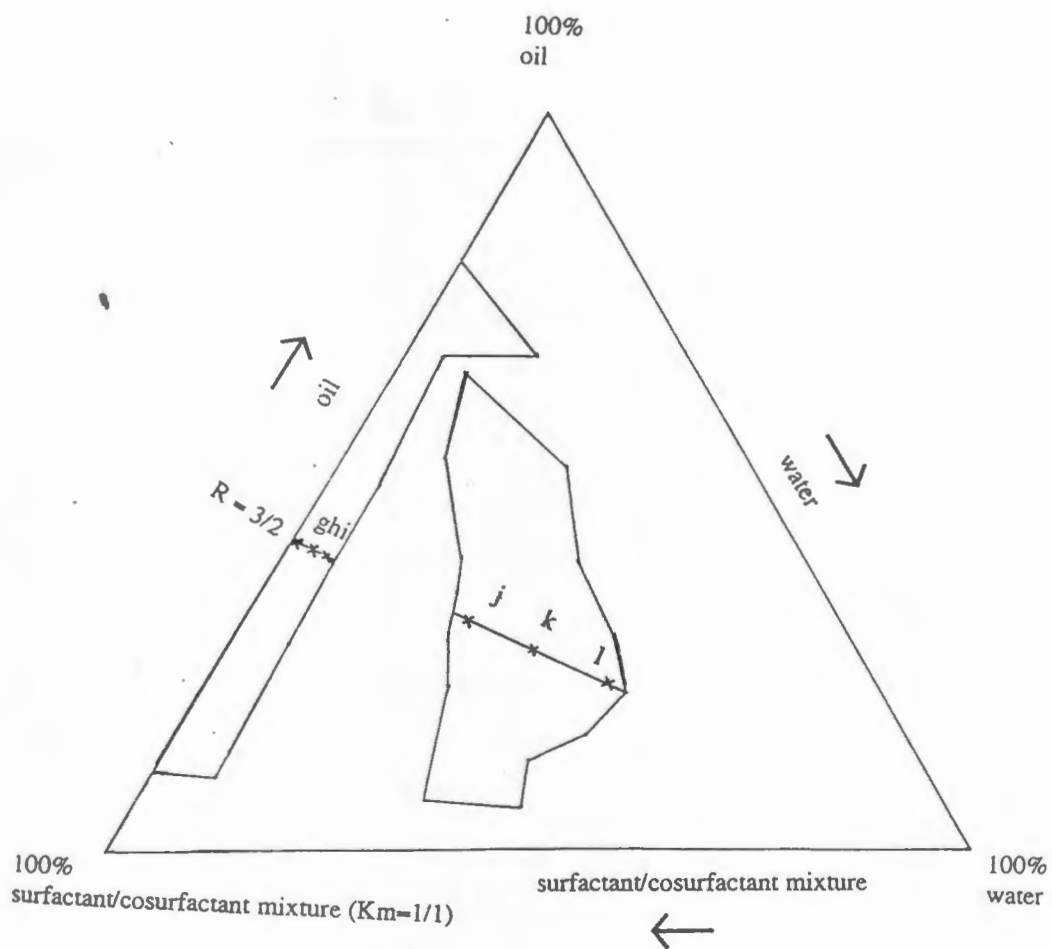


Fig. 4.1.1.D Phase diagram of soybean oil microemulsion at surfactant/cosurfactant ratio ( $K_m$ ) of 1/1 at 20°C.

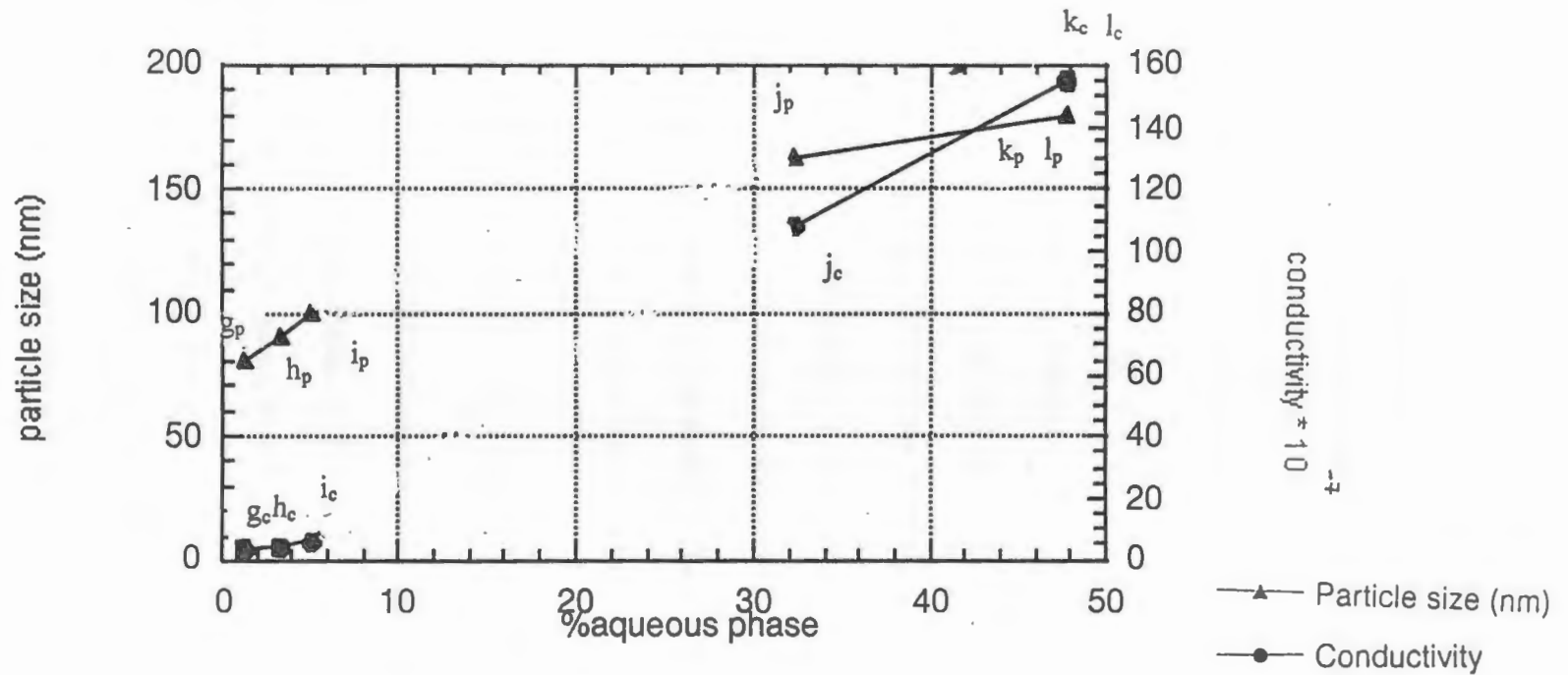


Fig. 4.1.2 Particle size and conductivity changes of the isotropic phases at increasing water content of the isotropic phases obtained at  $K_m = 1/1$  of Labrasol/Plurololeique CC 497 and mineral oil combinations.

(points g,h,i in Fig. 4.1.2), the system is likely to be a w/o emulsion. The low conductivity values obtained ( $3.12\text{-}5.98 \times 10^{-7} \Omega^{-1}$ ) strengthen this fact. In this region the particles measured are around 80-100 nanometers. These points correspond to point g to i in Fig. 4.1.1.D indicating that at the oil/surfactant corner, the system is in the form of w/o emulsion.

The second isotropic phase obtained in the middle is also investigated by measuring the conductivity and particle sizes at the surfactant/oil/water combinations of j, k, l in Fig. 4.1.1.D. Along this region the particle size increases from 143 to 180 nanometers. At the same time, the conductivity readings increase ten times from  $8.76 \times 10^{-6}$  to  $1.55 \times 10^{-5} \Omega^{-1}$ , which may indicate particle aggregation. This region is likely to be an o/w microemulsion. When water content increases above 48.82% (w/w) water the system turns turbid and forms an o/w emulsions. As shown in Fig. 4.1.1.d, the largest isotropic phase occurs at Km of 3/2 (HLB 10.8), where the system is able to solubilize the maximum amount of water (54.55%).

The surfactant/cosurfactant combination corresponding to (Km) of 3/2 also provides the largest isotropic area. The system is enlarged in Fig. 4.1.1.E. The changes in the particle size and conductivity of the isotropic phase occurring at at Km = 3/2 combination were also investigated along the points a, b, c, d, e, and f.

The particle sizes obtained in Km = 3/2 combination are similar to Km = 1/1 combination at the oil-surfactant line, which were 100 and 110 nm. Increasing particle size to 220 nm

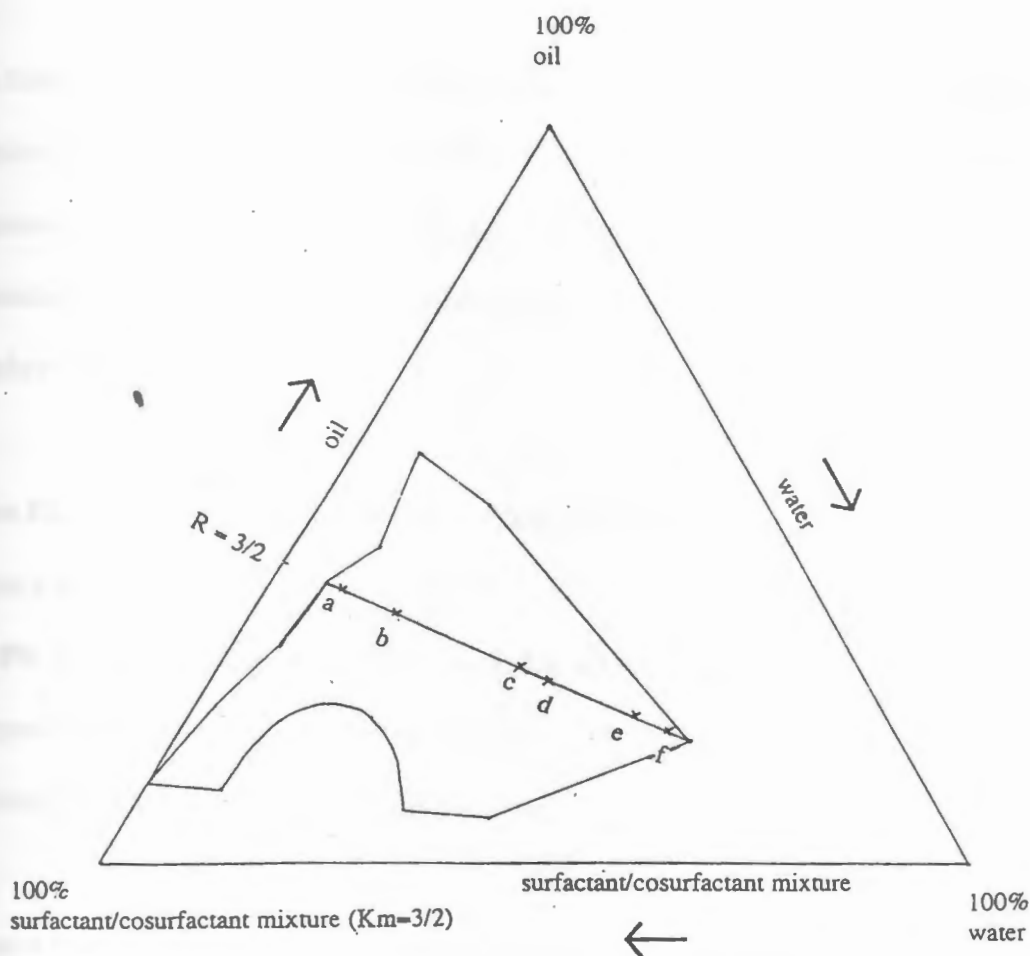


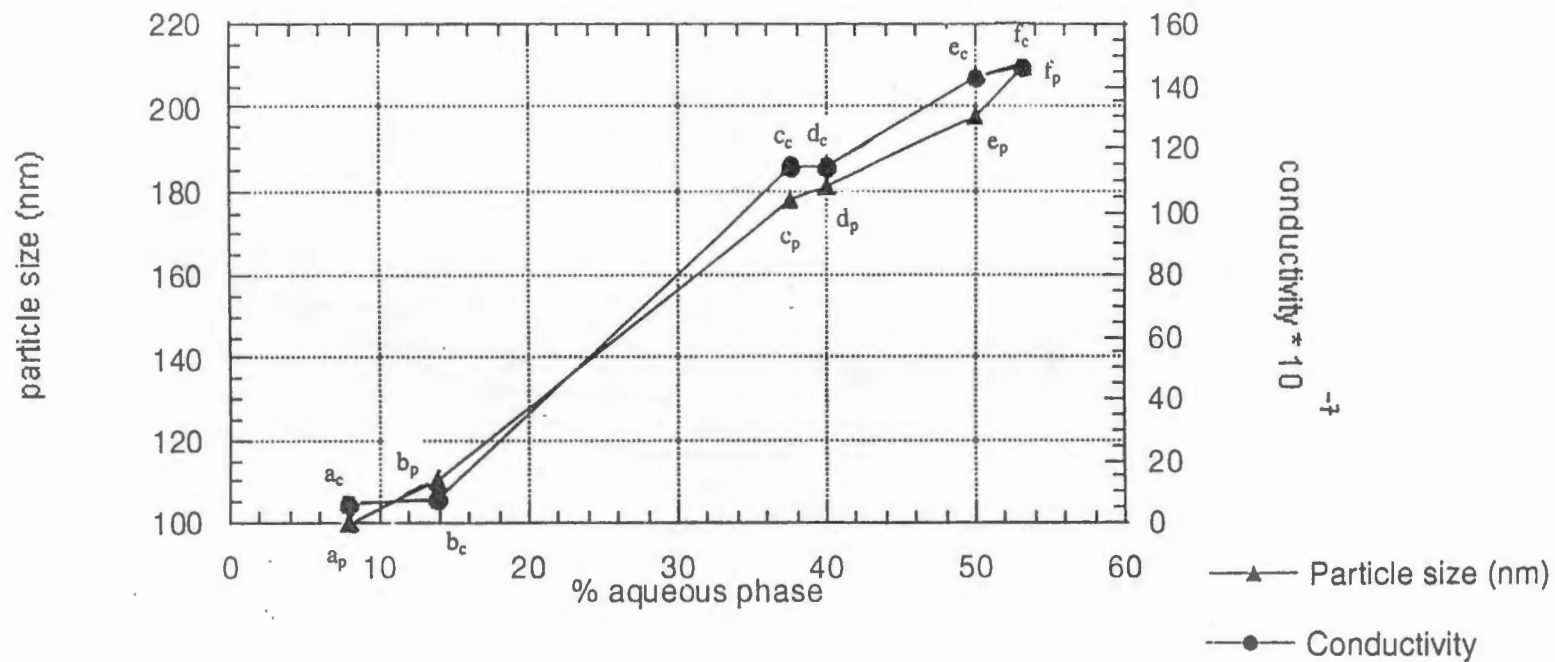
Fig. 4.1.1.E Phase diagram of mineral oil microemulsion at surfactant/cosurfactant ( $K_m$ ) =  $3/2$  at  $20^\circ\text{C}$ .

may indicate increased water uptake and it is almost linearly dependent on the water content. However, upon increasing water concentration by 4 fold, the conductivities jumped from  $7.30 \times 10^{-7}$  to  $1.46 \times 10^{-5} \Omega^{-1}$ , Fig. 4.1.3.

The findings may not necessarily indicate a phase transformation of o/w. If it were so, particle size increase would not follow this finding. However, Fig.4.1.3 may indicate an increased interaction of the water droplets, transformation to a bicontinuous phase or formation of a cubic phase. Similar phenomena have been discussed by Joubran<sup>31</sup>, Fletcher and Morris.<sup>39</sup>

When HLB of the surfactant/cosurfactant mixture increases from 10.8 to 12.4, a thin o/w region appears which can solubilize up to 15.22% oil. In Fig.4.1.1(f), a maximum of 60.88% surfactant mixture is needed to have o/w microemulsions at specified compositions. In the absence of Plurololeique CC 497, Labrasol cannot form any isotropic domains with mineral oil, Fig. 4.1.1(g).

Figure 4.1.4 demonstrates the effects of surfactant/cosurfactant ratios on the extent of isotropic regions that were obtained with soybean oil. Similar to the isotropic phases obtained with mineral oil neither the surfactant nor the cosurfactant can form isotropic regions by themselves, Fig. 4.1.4(a) and 4.1.4(g).



4.1.3 Particle size and conductivity changes of the isotropic phases at increasing water content of the isotropic phases obtained at  $K_m = 3/2$  of Labrasol/Plurololeique CC 497 and mineral oil combinations.

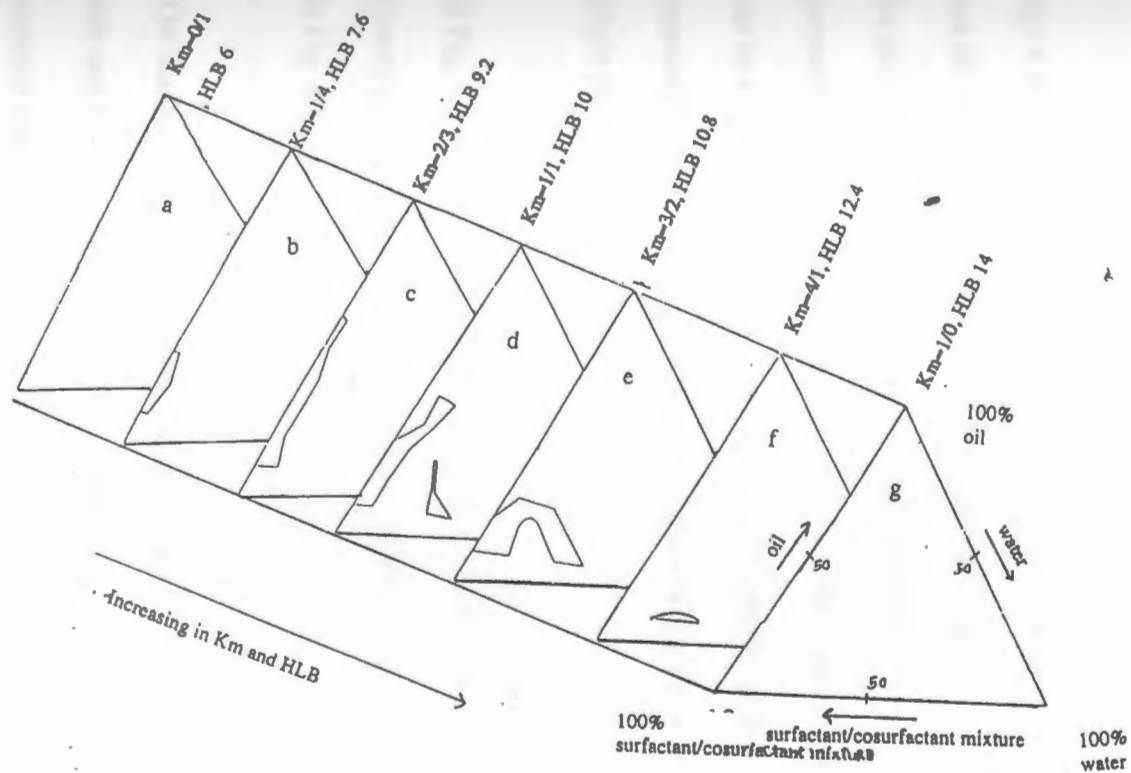


Fig. 4.1.4 The effect of Labrasol/Plurololeique CC 497 ratios on isotropic domains of soybean oil at 20°C.

At a high Plurololeique CC 497 ratio of  $K_m = 1/4$ , Fig. 4.1.4.(b), there is a very thin isotropic region that appears between 9.75% oil to 29.24% oil concentrations. When Labrasol concentration is increased ( $K_m = 2/3$ ), more oil at the surfactant-oil line can be solubilized (58.82% oil), Fig. 4.1.4.(c). The isotropic regions obtained with soybean oil are smaller than the ones that are obtained with mineral oil. However at  $K_m = 1/1$ , the same pattern of phase separations similar to Fig. 4.1.1(d) occurred.

Fig. 4.1.4.D is in the enlarged form of 4.1.4.d. The possible structures of the points ghi and jkl combinations were further investigated. Fig. 4.1.5 shows particle size changes of the ghi combination along the ghijkl line. The particle size appears to remain constant at increasing water content. It is almost twice of the mineral oil systems. These systems can be w/o microemulsions or oil continuous lamellar phase. Their polydispersity remains the same at increased water content which may indicate a uniform homogeneous single phase w/o emulsion.

In Fig. 4.1.4(e), the  $K_m = 3/2$  combination is demonstrated. At this combination, the system demonstrates a horse-shoe shaped isotropic region, which is shown in large scale in Fig. 4.1.4.E.

The maximum amount of water that this system can solubilize is 34.81%. The particle size and conductivity was studied along the a-f line, Fig. 4.1.6. The conductivity still remains very low ( $2.8 \times 10^{-7} \Omega^{-1}$ ) and the particle size remains around 182 nanometers. This microemulsion is likely to be a w/o type. Both properties remain constant upon



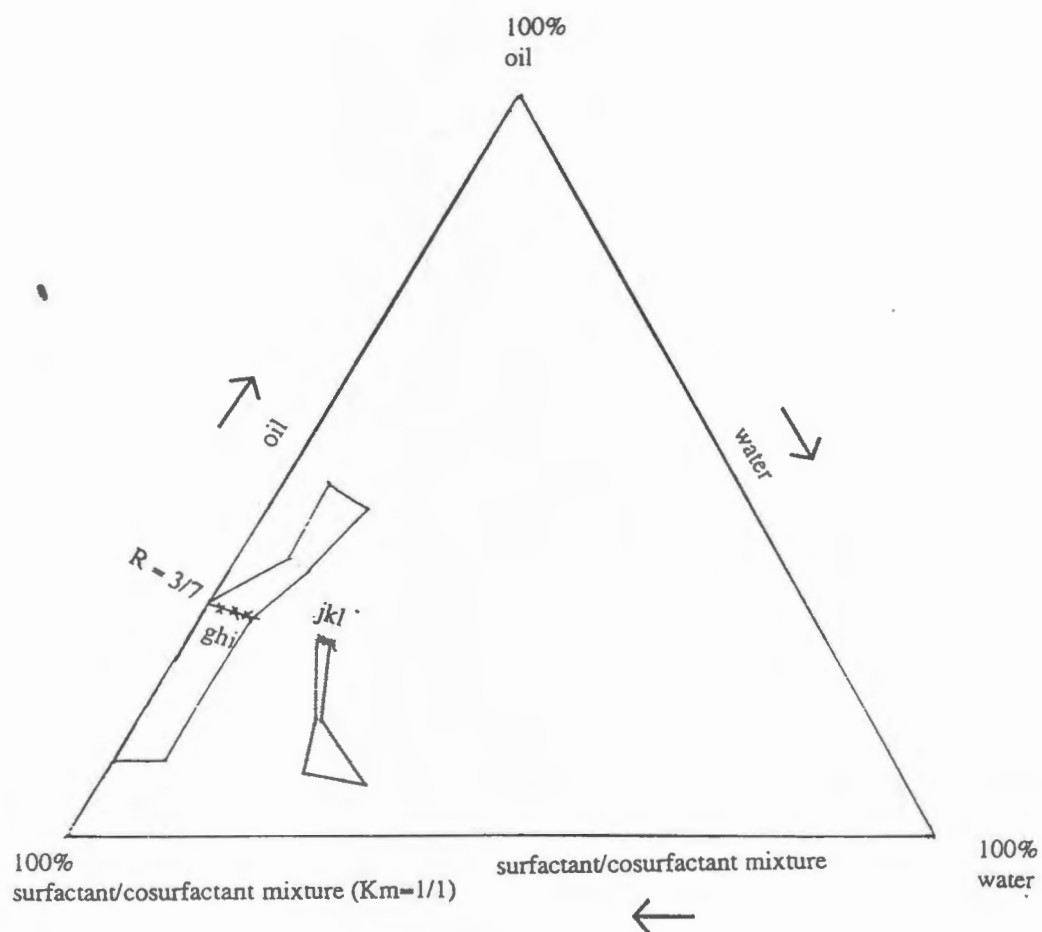


Fig. 4.1.4.D Phase diagram of soybean oil microemulsion at surfactant/cosurfactant ratio ( $K_m = 1/1$ ) at 20°C.

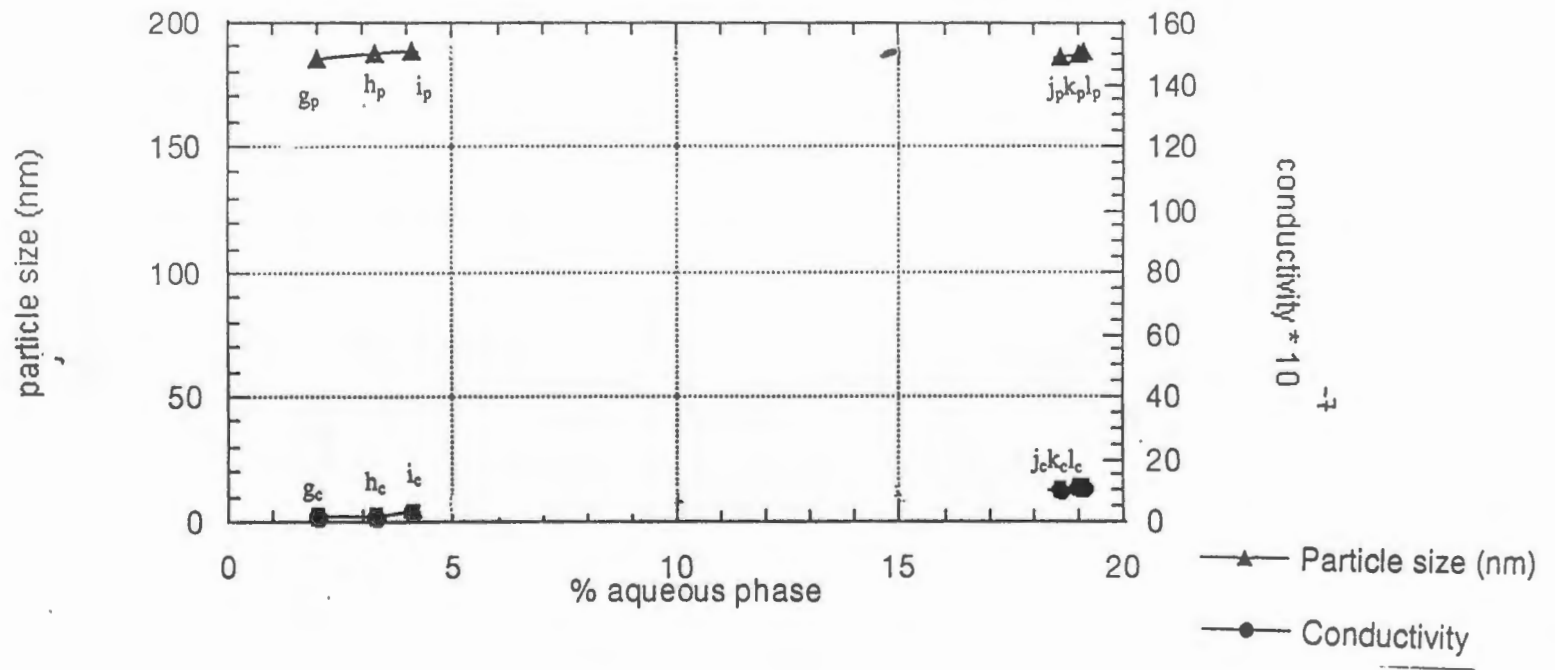


Fig. 4.1.5 Particle size and conductivity changes of the isotropic phases at increasing water content of the isotropic phases obtained at  $K_m = 1/1$  of Labrasol/Plurololeique CC 497 and soybean oil combination.

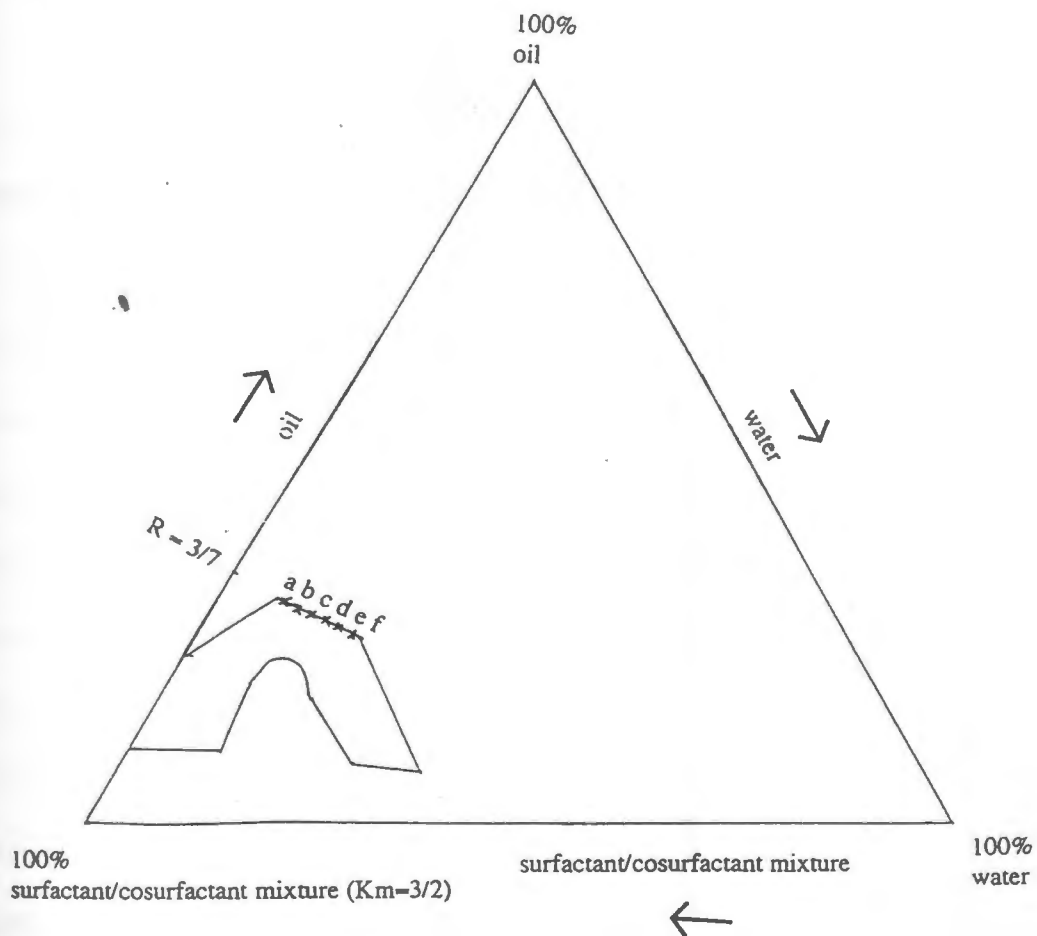


Fig. 4.1.4.E Phase diagram of soybean oil microemulsion at  
 surfactant/cosurfactant ( $K_m$ ) =  $3/2$  at  $20^\circ\text{C}$ .

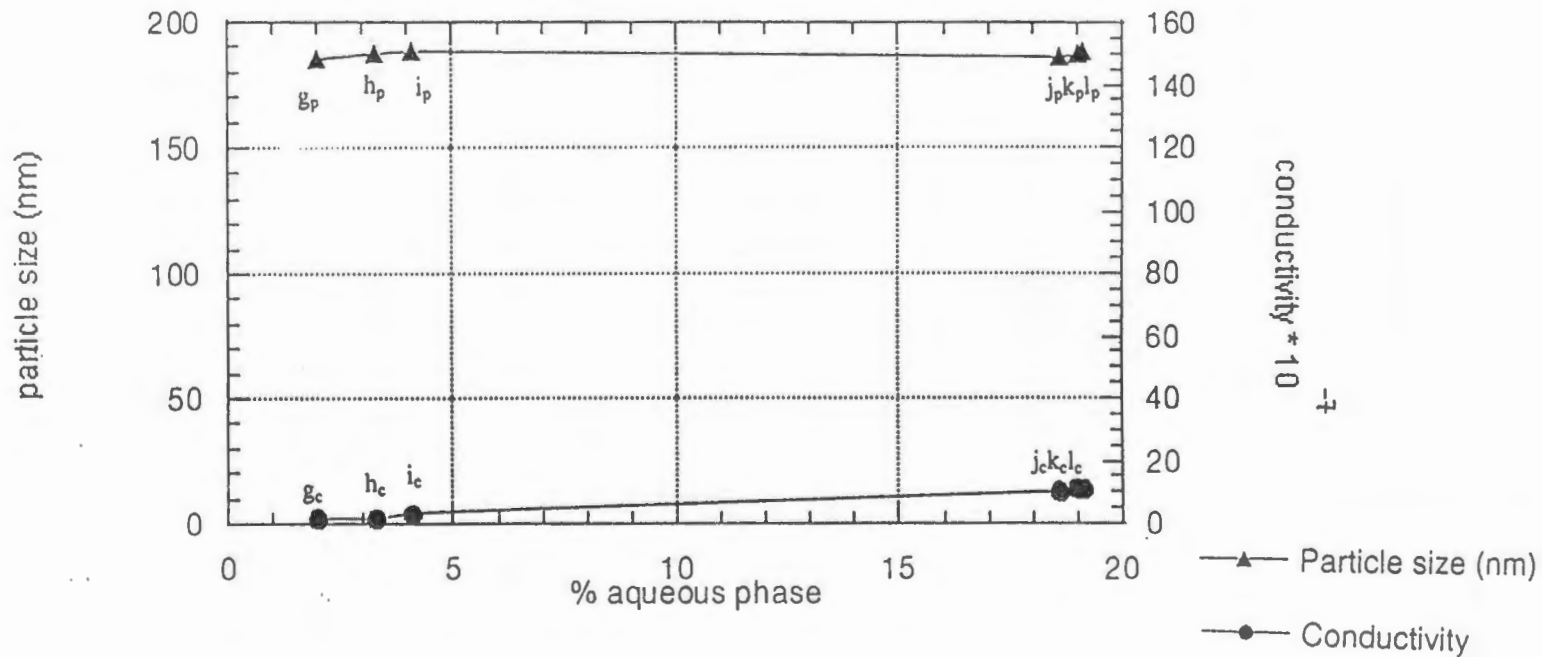


Fig. 4.1.6 Particle size and conductivity changes of the isotropic phases at increasing water content of the isotropic phases obtained at  $K_m = 3/2$  of Labrasol/Plurololeique CC 497 and soybean oil combinations.

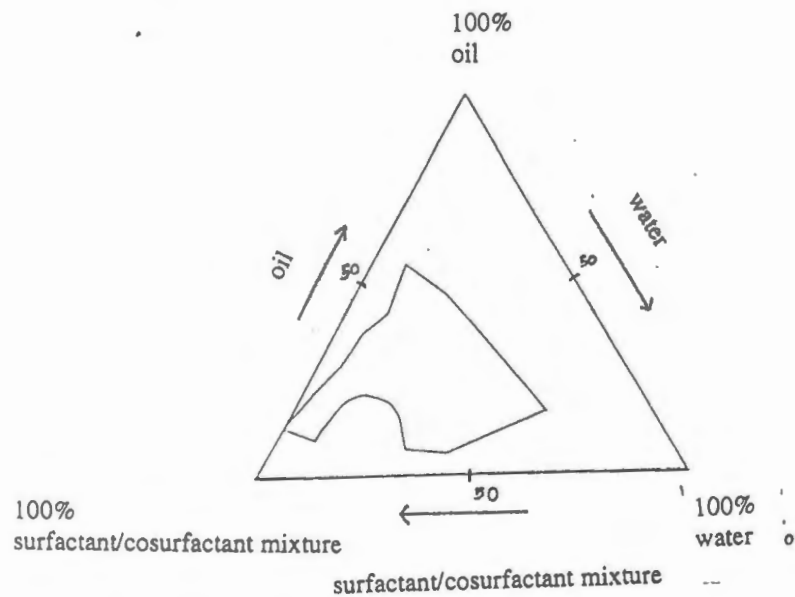
increasing water content. Since the particle size remains constant up to 20% water concentration region, it is expected that the number of the water droplets in oil increase until the whole system reverses into a macroemulsion.

## 4.2 Effect of Oil Type and Polarity

In this section, the influence of oil polarity (soybean oil, superpurified soybean oil and mineral oil) on microemulsion formation was investigated.

The phase behaviors of Labrasol-Plurololeique CC 497 mixture ( $K_m = 3/2$ ) with light mineral and soybean oil is demonstrated at 25 °C in Fig. 4.2.1. It is found that the surfactant/cosurfactant system used favors the light mineral oil which is a nonpolar hydrocarbon oil to soybean oil. This experiment supported the previous findings of Alander and Warenheim<sup>50</sup> who have compared triglycerides used in the microemulsions with ordinary hydrocarbons, and found that it is more difficult to solubilize triglyceride oils into microemulsions than hydrocarbons; eventhough the carbon chain length of soybean oil (18) is smaller than light mineral oil (15-25).<sup>55</sup> Alander and Warenheim<sup>50</sup> demonstrated that the polarity of the oils used in their study is a main factor influencing isotropic domains and solubilization of water and oil. With light mineral oil, o/w emulsions were obtained, whereas soybean oil demonstrated the presence of oil continuous systems which were not influenced by the increasing water concentration of the mixture.

A) Light mineral oil



B) Soybean oil

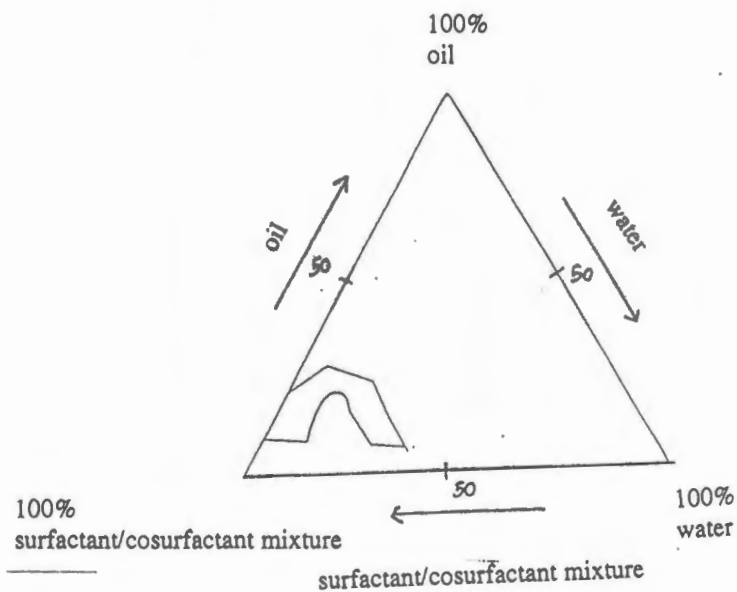


Fig. 4.2.1 The isotropic domains obtained using Labrasol/Plurololeique CC 497 3/2 mixture, light mineral oil, soybean oil and water at  $T = 25^{\circ}\text{C}$ .

By using a  $C_{12}E_{10}$  surfactant, Malcolmson and Lawrence<sup>14</sup> found that with the decreasing molecular weight of the oils, the formation of microemulsions was favored. These findings were also supported by the study of Aboofazeli et al.<sup>19</sup> who found that the smaller oils such as ethyl octanoate, ethyl oleate are preferential to soybean oil in the formation of a microemulsions. With the surfactant combination used in this study, the size of the oil appears to be less sensitive than its polarity.

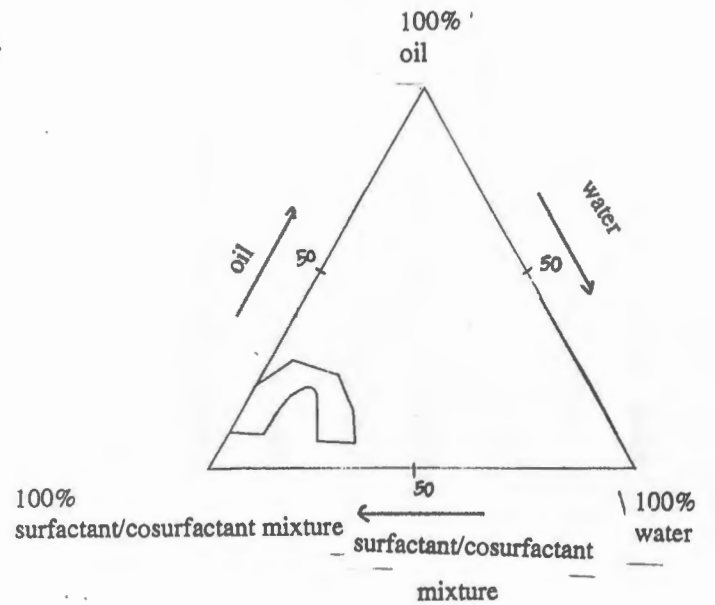
The superpurification of soybean oil has not affected the isotropic regions. According to the manufacturer, in the soybean oil (USP), the maximum polar impurities were 2.3-4.8% whereas in the superpurified soybean oil, this amount was reduced to less than 1%<sup>51</sup>

It was found that polar impurities present in soybean oil have not influenced the size of the isotropic domains obtained with superpurified soybean oil (Fig.4.2.2.). Malcolmson and Lawrence<sup>14</sup> also compared the effect of soybean oil and superpurified soybean oil using  $C_{18-1}E_{10}$  surfactant in microemulsion domains. The isotropic area was not found to change significantly. The maximum oil and water solubilized in soybean microemulsion are 28.85% oil and 34.81% whereas in superpurified soybean microemulsion, 28.30% oil and 30.27% water are solubilized.

#### 4.2.1 Influence of unsaturation of the C18 fatty acids

The phase behavior of mineral oil in the presence of 1 and 10% oleic, linoleic, and linolenic acids respectively, Labrasol-Plurololeique CC 497 mixture ( $K_m = 3/2$ ) and

A) Soybean oil



B) Superrefined soybean oil

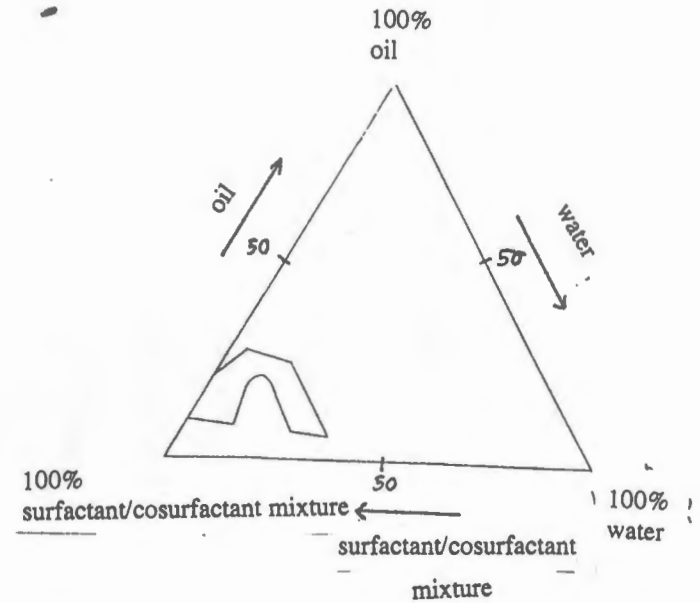


Fig. 4.2.2 Effect of superpurification of soybean oil on the isotropic domains of Labrasol/Plurololeique CC 497 ratio 3/2 mixture ( $K_m = 3/2$ ) isotropic phases at  $T = 25^\circ\text{C}$ .



distilled water at 25 °C is demonstrated in Fig. 4.2.3.

Oleic acid has one, linoleic has two, and linolenic acid has three double bonds. Their effect in the boundaries of the isotropic zone were studied by adding individual acids to mineral oil at 1-10% concentration.

At 1% concentration, all fatty acids have the same effect on the microemulsion domains of the mineral oil, Fig. 4.2.3. When the concentration of fatty acids increases to 10%, linolenic acid demonstrates the most notable change on the microemulsion domain whereas oleic acid has the least impact.

Incorporation of linolenic acid reduced the solubilization of the oil in the system. It appears that for C<sub>18</sub> fatty acids, more than the double bonds that they have in their structure, the amount incorporated in the oil phase influences microemulsion formation.

The same figure demonstrates that at 1% concentration, the fatty acid concentration does not influence the system, whereas at 10%, they interfere with the formation of isotropic domains immensely, because they increase the polarity of mineral oil. Therefore it is concluded that the concentration of the polar groups is the main factor influencing isotropic phase formation.

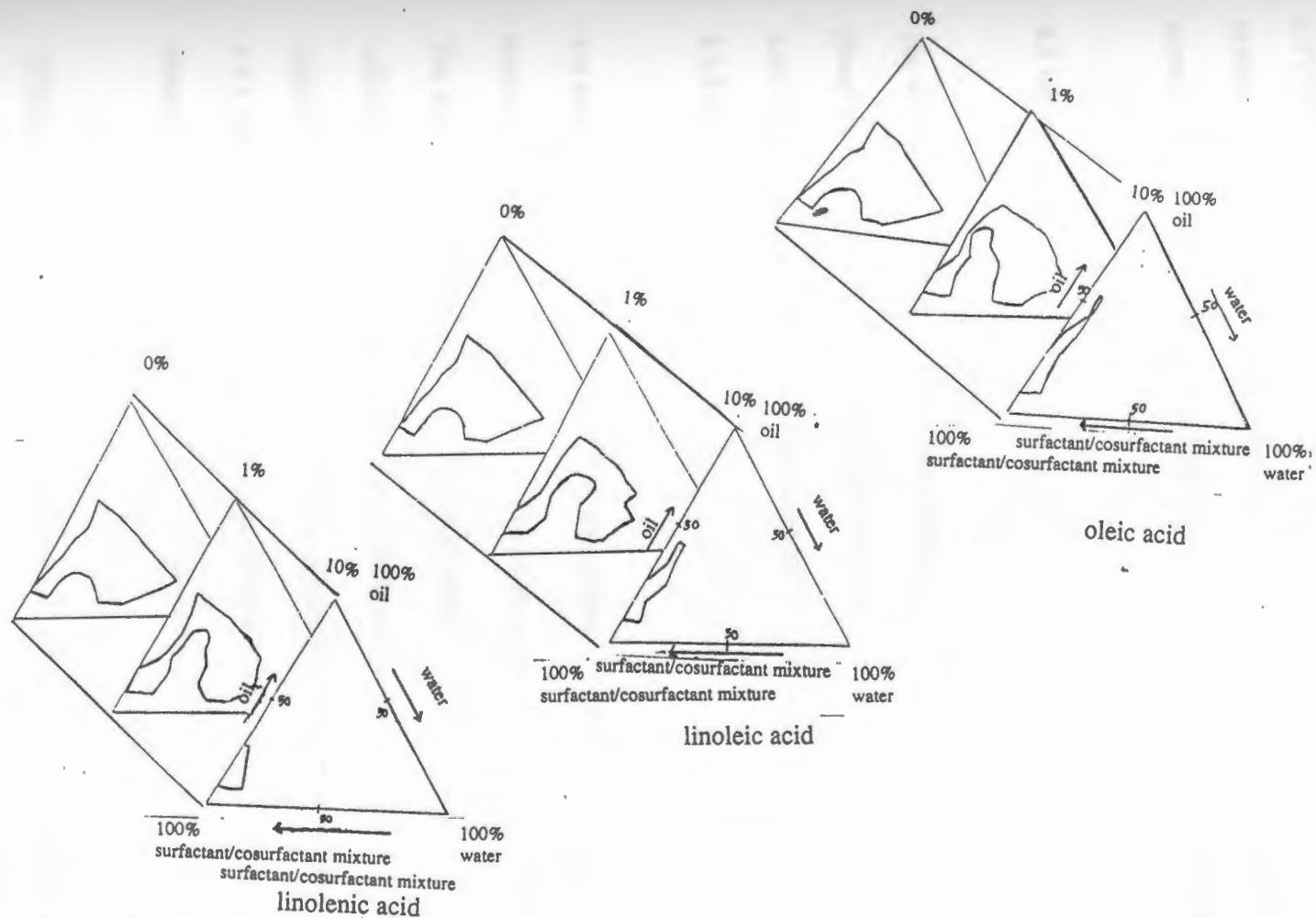


Fig. 4.2.3 Effect of fatty acids (oleic acid, linoleic acid, linolenic acid) on the isotropic domains of mineral oil at Labrasol/Plurololeique CC

497 ratio of 3/2,  $T = 25^{\circ}\text{C}$ .

Although no similar comparison appeared in the literature, comparison of the effects of soybean oil and Miglyol 812 (oil) made by Malcolmson and Lawrence<sup>14</sup> showed that the system which contained Miglyol 812/C18-1E10/water provided lower oil incorporation capacity than soybean oil system, because Miglyol 812 was more polar than soybean oil.

### 4.3 Influence of Temperature

The effect of temperature on the isotropic domains were investigated by determining phase behavior of the mixtures of mineral oil and soybean oil with surfactant and water at  $K_m = 1/1$ . The temperature were studied at 20°C, 25°C, 32°C and 37°C, Fig. 4.3.1 and 4.3.2 respectively.

For both oils, when  $K_m$  is 1/1 (HLB10), the system is extremely temperature sensitive between 20-25°C, Fig. 4 3.1 and 4.3.2. This is the same regardless of the oil type used. The hydrophilicities of Labrasol and Plurololeique CC 497 must be primarily influenced within this temperature range. When the temperature is increased to 25°C, the wide region of water continuous phase disappears entirely in the mineral oil systems in Fig. 4.3.1. This must be due to the dehydration of Labrasol, knowing that when the temperature increases the hydrophilicity of the surfactant decreases.<sup>3,13</sup>

Similarly, the small isotropic region occurring in the middle of the three phase diagram of soybean oil shown in Fig. 4.3.2 disappears when the temperature is increased to 32°C. At the surfactant-oil corner, the isotropic phase obtained appears to be temperature

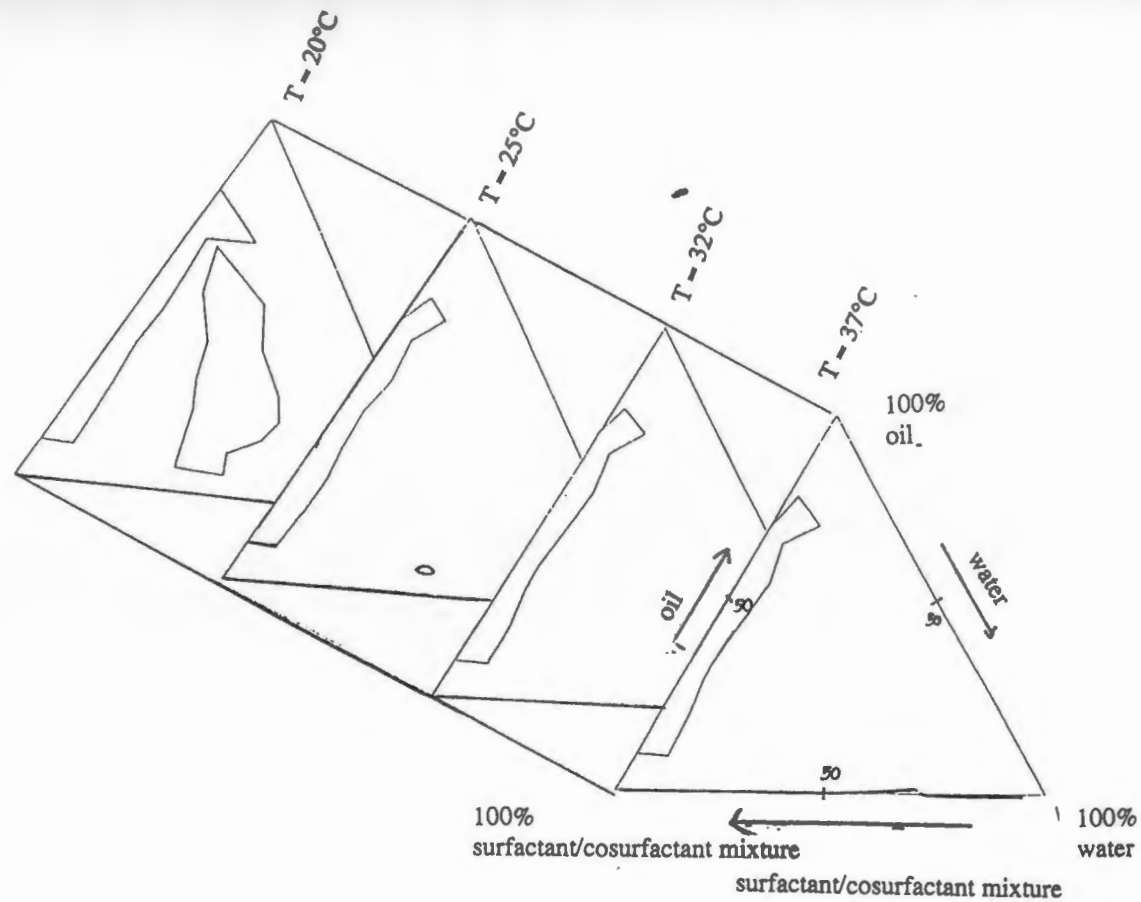


Fig. 4.3.1 Effect of the various temperatures on the isotropic domains of mineral oil at Labrasol/Plurololeique CC 497 ratio 1/1.

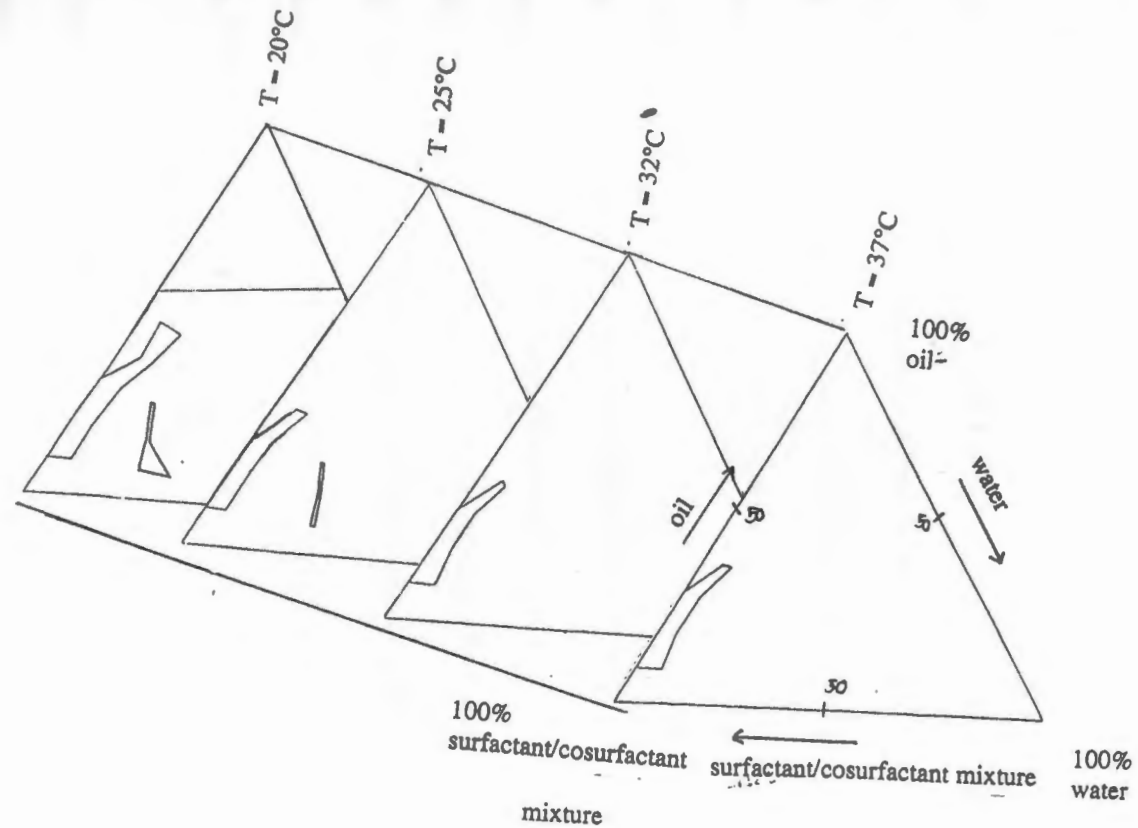


Fig. 4.3.2 Effect of the various temperatures on the isotropic domains of soybean oil at Labrasol/Plurololeique CC 497 ratio 1/1.

insensitive between 20-37°C indicating that this phase may be a micellar dispersion of the surfactant/cosurfactant in the oils studied, although there is about 7% water solubilized at this corner.

At compositions corresponding to  $K_m = 3/2$ , Fig. 4.3.3 for mineral oil microemulsion and Fig.4.3.4 for soybean oil microemulsions, temperature sensitivity of the systems is less and the critical temperature moves to 32-37°C region. These changes are expected because the cloud-points of the surfactants increase with increasing concentrations.<sup>60</sup>

At  $K_m = 3/2$ , the amount of Labrasol increases and therefore the hydrophilicity of the mixture increases. It can be concluded that within this particular mixture at this surfactant concentration, increasing the hydrophilicity reduces the temperature sensitivity of the surfactant mixture. Even in the 32-37°C region, water uptake is not significantly reduced (from 54.55% at 20°C to 44.44% at 37°C for mineral oil microemulsion and from 34.81% at 20°C to 32.52% at 37°C for soybean oil microemulsion ).

The compositions given in Fig.4.3.3 appear to be highly useful for topical applications because of their long term stability. They will not revert to emulsions provided that they remain within the same oil-surfactant-water composition.

For soybean oils, Fig.4.3.4, the temperature effect is not very critical and still allows the presence of a small island of isotropic phase in the middle of the diagram which contains 24.01-32.52% water.

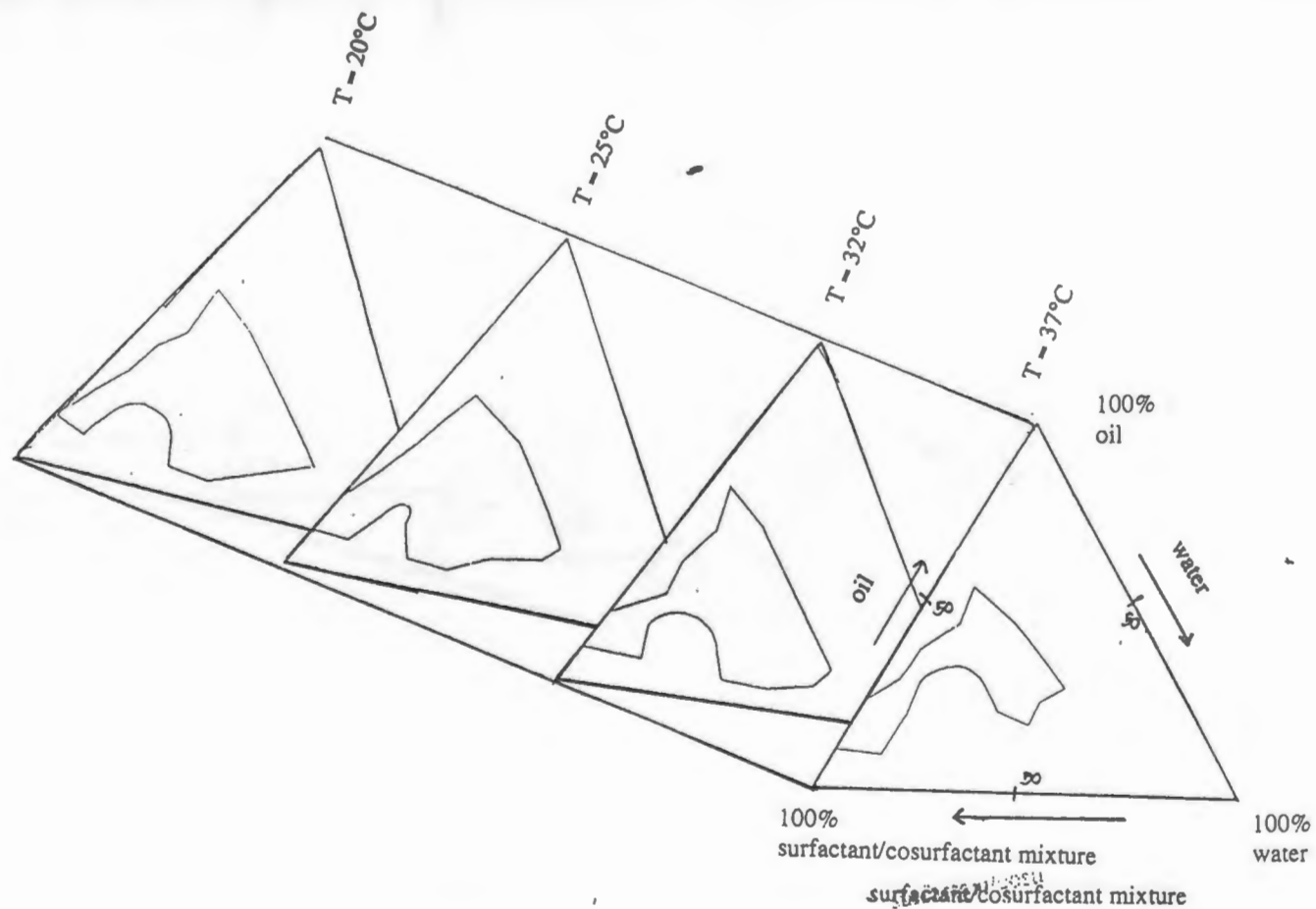


Fig. 4.3.3 Effect of the various temperatures on isotropic domains of mineral oil at Labrasol/Plurololeique CC 497 ratio of 3/2.

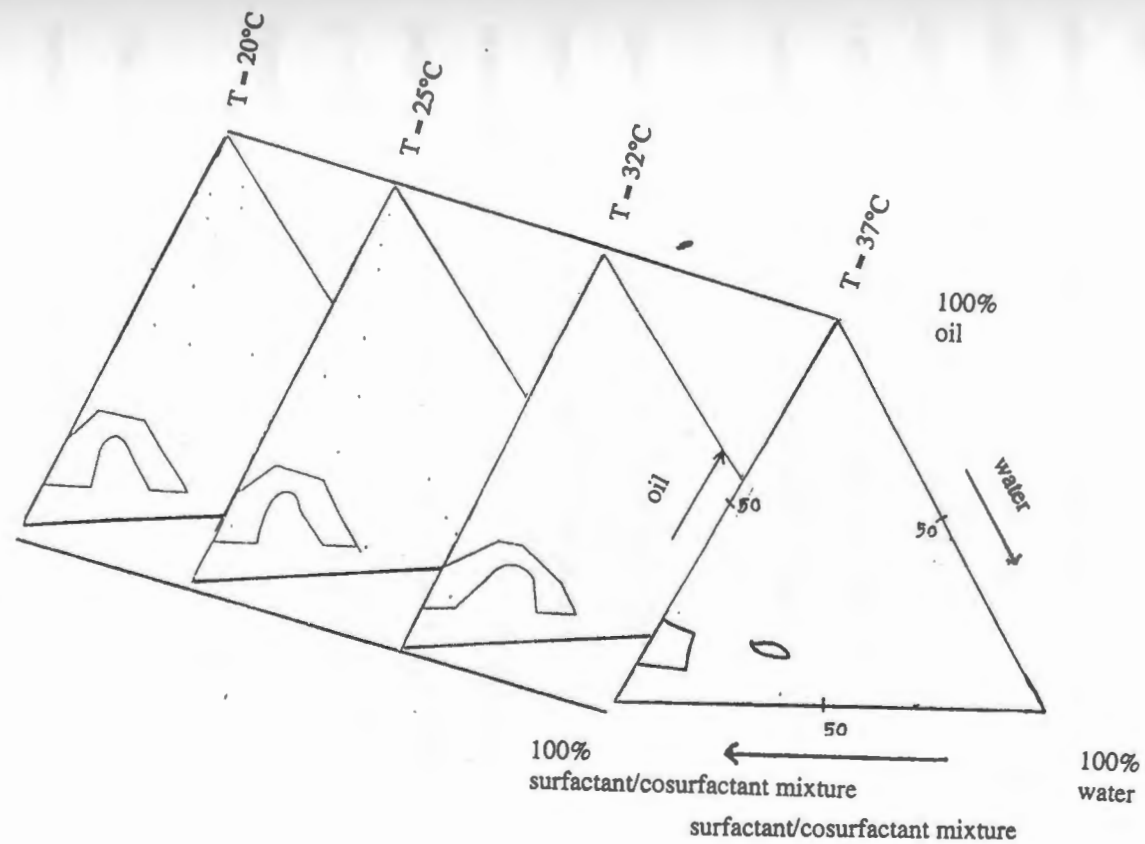


Fig. 4.3.4 Effect of the various temperatures on the isotropic domains of soybean oil at Labrasol/Plurololeique CC 497 at a Km ratio of 3/2.



#### 4.4 Effect of Aqueous Phase

The effect of the inorganic electrolytes on the isotropic domains was investigated by adjusting the ionic strength ( $\mu$ ) of the aqueous phase to 0.06 and 1 with NaCl and CaCl<sub>2</sub>. Effect of ions on the phase behavior of mineral oil and soybean oil systems was investigated at  $K_m = 1/1$  (Fig. 4.4.1 and 4.4.2) and  $3/2$  ratios (Fig. 4.4.3 and 4.4.4) at 25°C. The figures demonstrate that at  $\mu = 0.06$ , NaCl and CaCl<sub>2</sub> do not affect the isotropic regions of the mineral oil and soybean oil systems at  $K_m = 1/1$  and  $3/2$  ratios.

However at  $\mu = 1$  and  $K_m = 1/1$ , due to dehydration of the surfactants the small island present in the middle of the diagram disappears as the result of NaCl and CaCl<sub>2</sub> addition, as shown in Fig. 4.4.1 and 4.4.2 respectively. Dehydration effect of the salts is less pronounced at higher Labrasol concentrations, Fig. 4.4.3 and 4.4.4, with the exception of  $\mu = 1$  in the mineral oil emulsions. Dehydration effects of both NaCl and CaCl<sub>2</sub> are comparable with the temperature effects.

The results obtained are somewhat expected because the effect of the electrolytes on the nonionic surfactants is less than that on ionic surfactants, and nonionic surfactants are not affected as rapidly as the ionic ones by the dehydrating effects of the electrolytes.<sup>18</sup>

However, there are reports showing that the fluoride and hydroxide ions can break the structure of water around ionic heads of nonionic surfactants and cause instability of the microemulsion.<sup>60</sup>

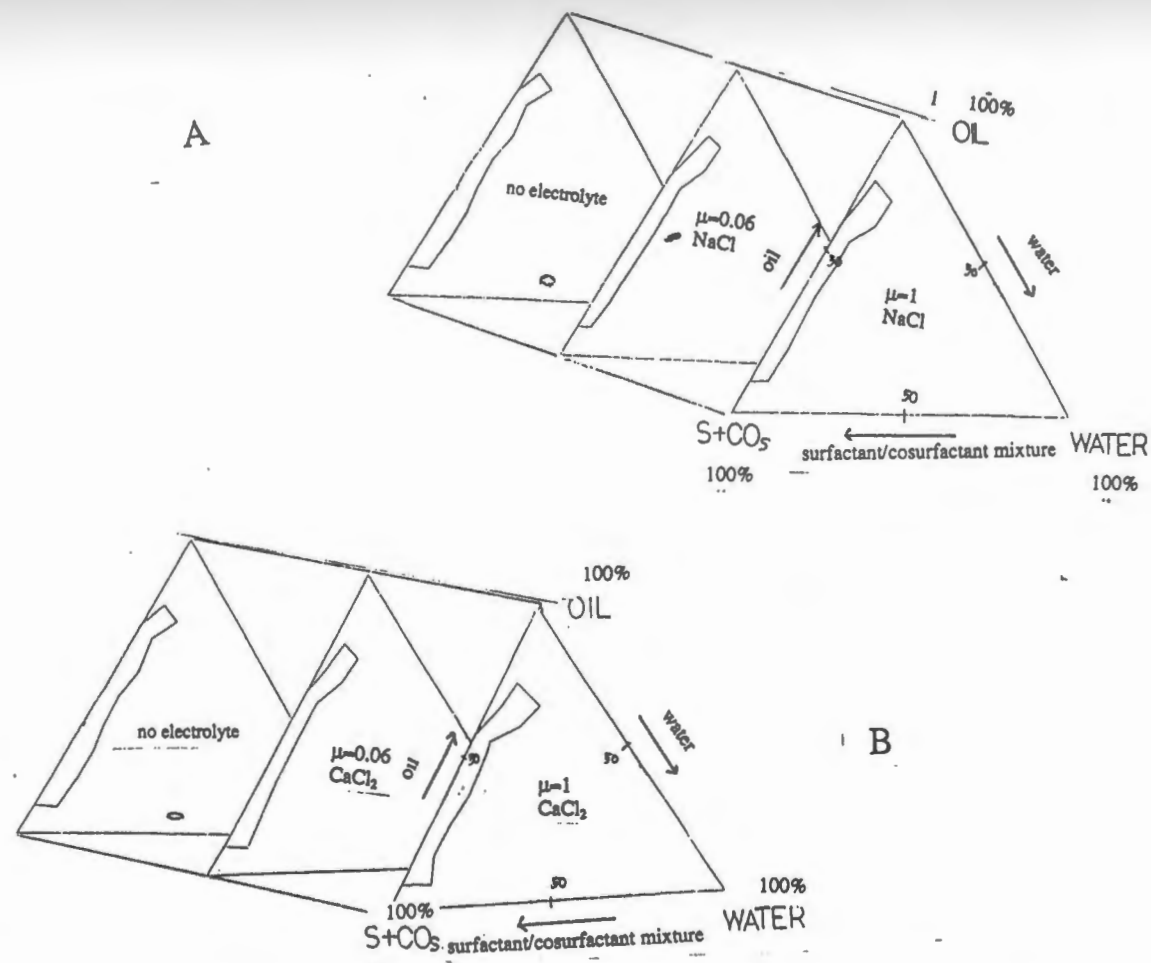


Fig. 4.4.1 Effect of electrolyte types and concentrations on isotropic domains of mineral oil at Labrasol/Plurololeique CC 497 ratio of 1/1,  $T = 25^{\circ}\text{C}$  in A: NaCl was used to adjust the ionic strength whereas in B, CaCl<sub>2</sub> was utilized.

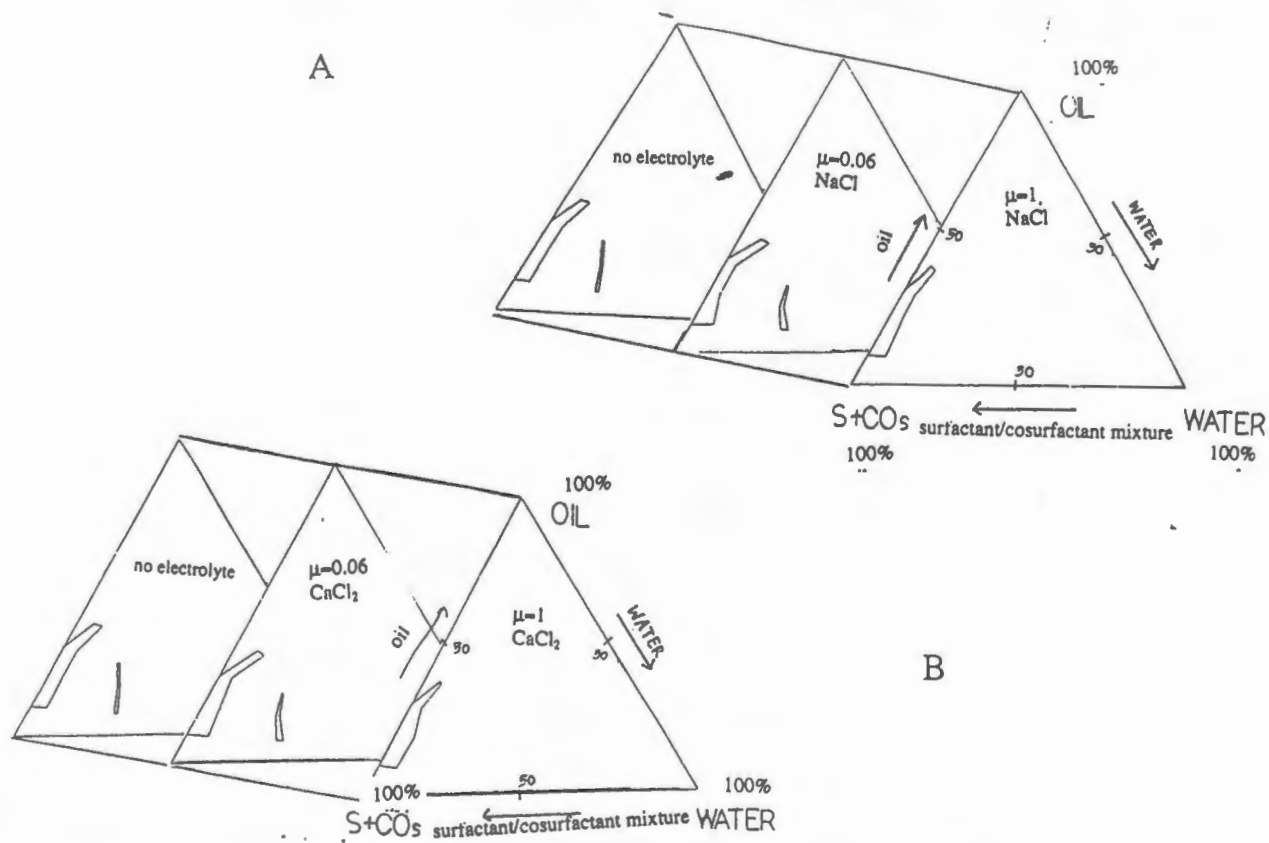


Fig. 4.4.2 Effect of electrolyte types and concentrations on isotropic domains of soybean oil at Labrasol/Plurololeique CC 497 ratio of 1/1,  $T = 25^\circ\text{C}$  in A: NaCl was used to adjust the ionic strength whereas in B,  $\text{CaCl}_2$  was utilized.

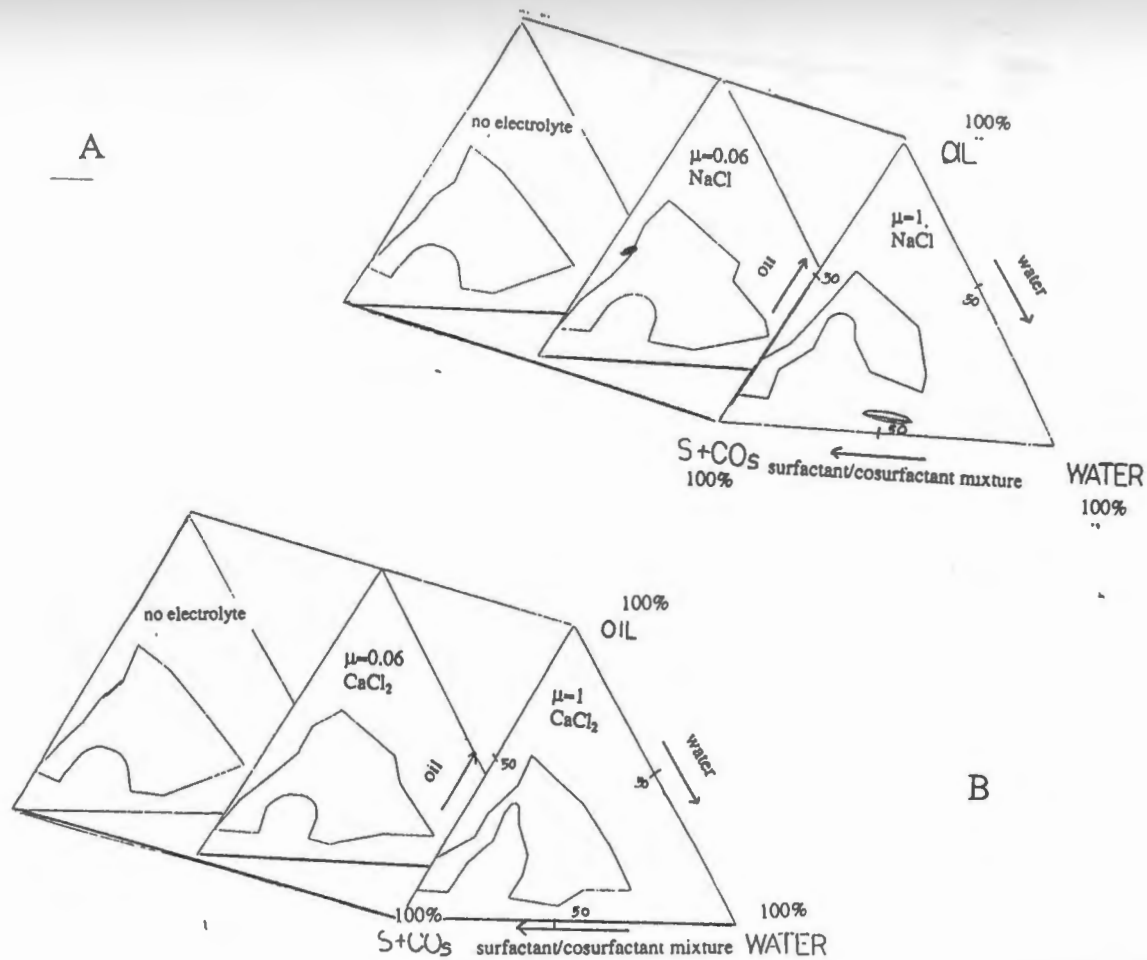


Fig. 4.4.3 Effect of electrolyte types and concentrations on isotropic domains of mineral oil at Labrasol/Plurololeique CC 497 ratio of 3/2,  $T = 25^{\circ}\text{C}$  in A: NaCl was used to adjust the ionic strength whereas in B, CaCl<sub>2</sub> was utilized.

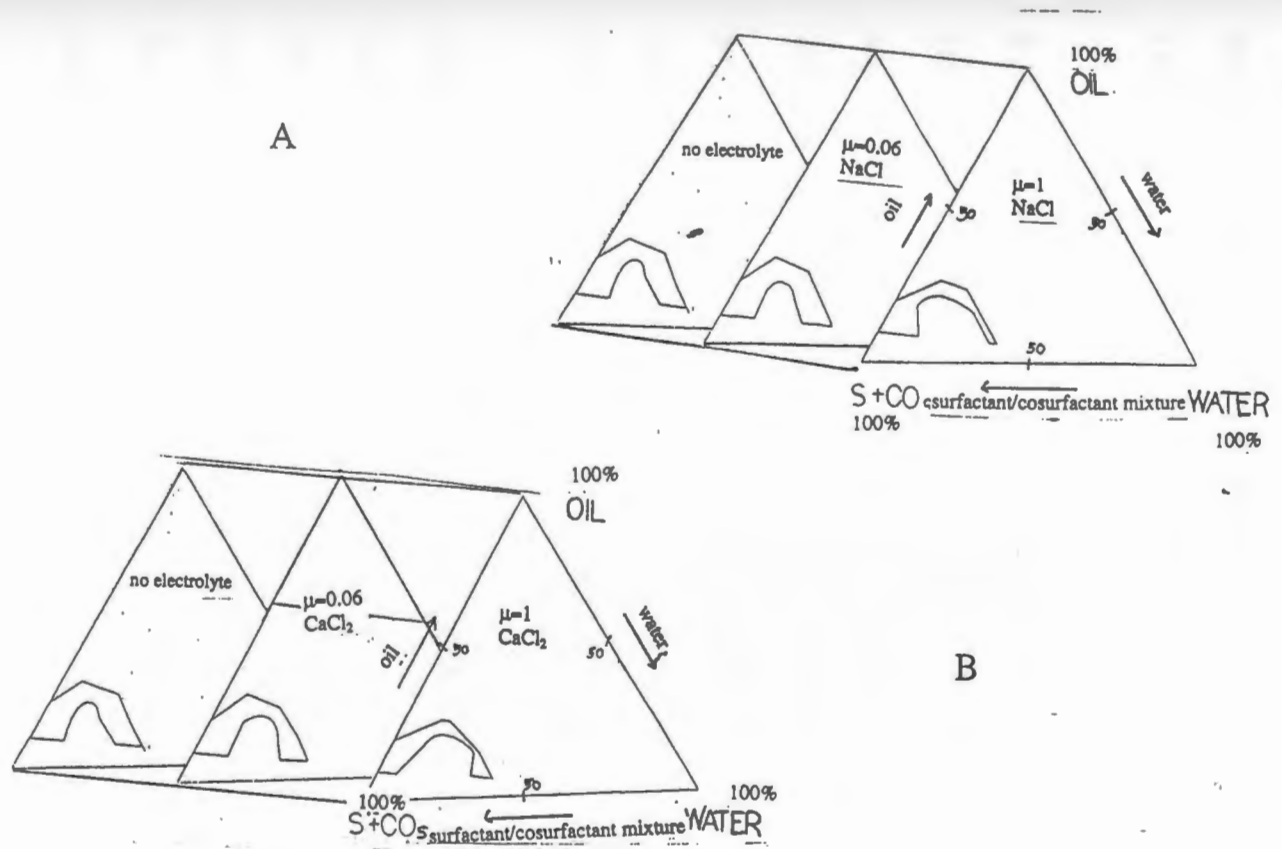


Fig. 4.4.4 Effect of electrolyte types and concentrations on isotropic domains of soybean oil at Labrasol/Plurololeique CC 497 ratio of 3/2,  $T = 3/2$ ,  $T = 25^\circ\text{C}$  in A: NaCl was used to adjust the ionic strength whereas in B,  $\text{CaCl}_2$  was utilized.

Kahlweit et al.<sup>79</sup> classified NaCl within a group that effectively caused salting out of the nonionic surfactants. However, within the region of the ionic strengths used, NaCl and CaCl<sub>2</sub> are not destructive for our systems, Figs. 4.4.1-4.4.4.

It is interesting to see that NaCl reduces the isotropic areas more effectively than CaCl<sub>2</sub>, Fig. 4.4.1-4.4.4. This can be explained as the result of the smaller size of sodium which readily dehydrates the polar heads of the surfactant than that of CaCl<sub>2</sub>.<sup>60</sup> As the ionic strength of the solution was decreased by the addition of NaCl and CaCl<sub>2</sub>, the effective polar part of the surfactant shrinks and becomes less bulky than the hydrophobic part. The HLB moves towards lower regions and as the result, the o/w structures are not favored<sup>13,18,61,62</sup>.

The pH effect was studied at Km = 3/2 surfactant combination, for both mineral oil and soybean oil systems. For the mineral oil microemulsions, the pH does not appear to be effective at 3/2 surfactant combination between pH of 1.5 to 7.4, Fig. 4.4.5. This finding offers an advantage for Labrasol-Plurololeique CC 497 systems for topical formulations. It is even more encouraging because the buffer systems used contained different acid/salt combinations. HCl, USP was used for pH 1.5, and phosphate buffer was used for pH 7.4.

Their effects were compared against that of distilled water. The excess H<sup>+</sup> ions do not influence the original isotropic nature of the mineral oil systems. Soybean microemulsion, shown in Fig. 4.4.6 also demonstrates the same pH stability.

This property may be specifically useful for small-volume intradermal or intramuscular injection dosage forms.

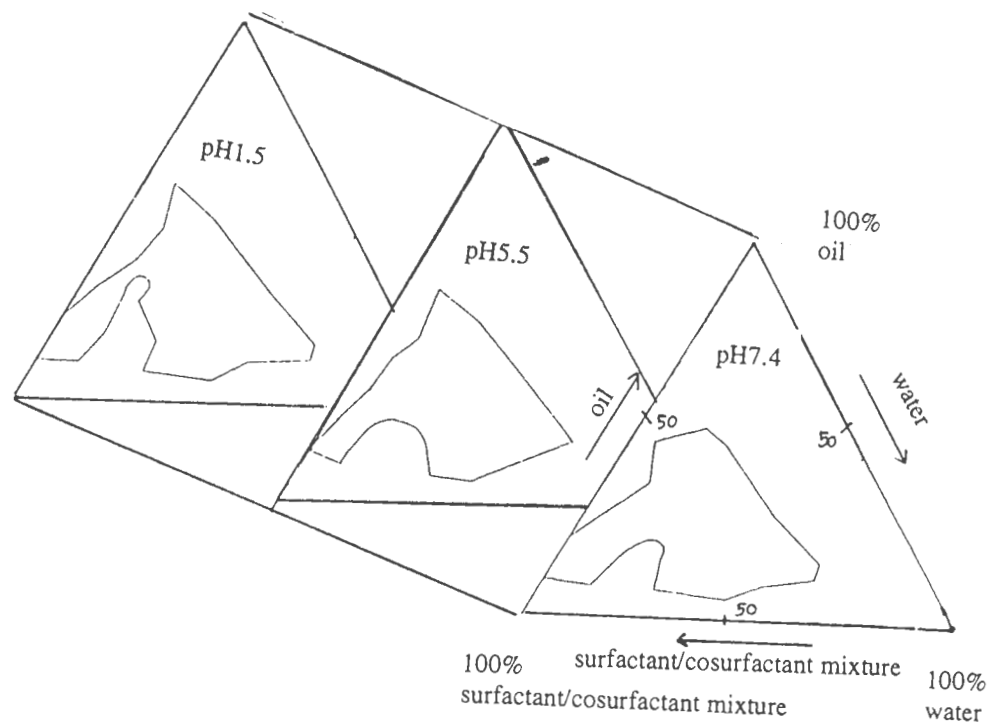


Fig. 4.4.5 Effect of the aqueous phase on the isotropic domains of mineral oil at Labrasol/Plurololeique CC 497 ratio of 3/2,  $T = 25^{\circ}\text{C}$ . (pH 1.5 and 7.4 were obtained by USP HCl and phosphate buffers, pH 5.5 was deionized water).



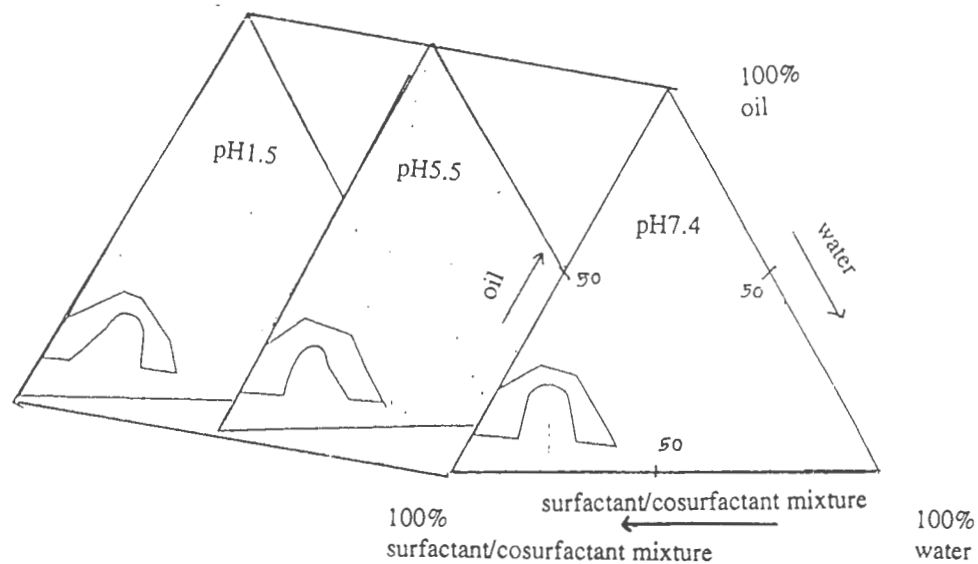


Fig. 4.4.6 Effect of pH of the aqueous phase on the isotropic domains of soybean oil at Labrasol/Plurololeique CC 497 ratio of 3/2,  $T = 25^{\circ}\text{C}$ . (pH 1.5 was obtained by USP HCl buffer, pH 5.5 was only deionized water, and pH 7.4 was obtained by USP phosphate buffer).

## 5. SUMMARY AND CONCLUSIONS

Although microemulsions have been widely investigated and applied in various industries, existing surfactant systems available for microemulsification have limited their applications in the pharmaceuticals and cosmetics. This work determined the boundaries of Labrasol and Plurololeique CC 497 combinations in microemulsion systems that may be useful as pharmaceutical and cosmetic delivery systems. It investigated the effects of surfactant/cosurfactant ratios, mineral oil and soybean oils, fatty acids, pH, electrolytes (NaCl and  $\text{CaCl}_2$ ), and temperature on the solubilization of oil and water phases.

The system provides the largest isotropic regions with mineral oil and soybean oil when a surfactant/cosurfactant combination of  $K_m = 3/2$  (HLB of 10.8) is used. The  $K_m$  of 1/1 (HLB = 10) is also found to be a useful combination. At HLB 10.8, the temperature sensitivity decreases and the critical temperature moves from 20-25°C to 32-37°C. Therefore, this surfactant/cosurfactant ratio gives an optimum HLB to be used in the microemulsion formulation. At this combination, the isotropic regions become less sensitive to the presence of NaCl and  $\text{CaCl}_2$  up to ionic strength of 1 ( $\mu=1$ ). The microemulsions prepared tolerate pH changes of the aqueous phase from 1.5 to 7.4. Mineral oil system provides a wider selection of oil-surfactant-water combination that can be used in microemulsion formulations than soybean oils systems. The latter is limited to solubilization of 28% oil and 34% water. All the systems are free-flowing in nature and suitable for injection.

We also demonstrated that the presence of polar fatty acids (oleic acid, linoleic acid, and linolenic acid) in a nonpolar oil (mineral oil in a low concentration (1%)) has not significantly affected the boundaries of the isotropic domains. Phase changes occur when their concentrations are increased to 10%, which shrinks the isotropic zones. This effect may be related to the concentration and number of double bonds present in the fatty acid system. There is a trend showing that increasing double bonds in the fatty acids reduces the microemulsion domains.

## 6. REFERENCES

1. Usacheva, T.M., Lifanova, N.V., and Zhuralev, V.I., Dielectric Properties of Nonionic Microemulsions, Colloid Journal, **59**, 79-85, 1997.
2. Fox, C., Cosmetic Emulsions In: Emulsion and Emulsion Technology Part II. Surfactant Science Series, Marcel Dekker Press, New York, **6**, 1974.
3. Swarbrick, J., and Boylan, J., Microemulsions: Encyclopedia of pharmaceutical technology, **9**, 392-396, 1988.
4. Rosano, H., Cavallo, J.L, Chang, D., and Whittam, J.H., Microemulsions: A commentary on their preparation, J. Soc. Chem., **39**, 201-209, 1988.
5. Garcia-Celma, M.J., Solubilization of Drugs in Microemulsions in Industrial Applications of Microemulsions., Marcel Dekker, New york.
6. Constantinides, P., Lancaster, C.M., Marcello, J., Chiossone, D.C., Orner, D., Hidalgo, I., Smith, P., Sarkahian, A., Yiv, S.H., and Owen, A., Enhanced intestinal absorption of an RGD peptide from water-in-oil microemulsions of different composition and particle size, J. of Controlled Release, **34**, 109-116, 1995.
7. Dangi, J.S., Vyas, S.P., and Dixit, V.K., The role of mixed micelles in drug delivery. I. Solubilization. Drug Development and Industrial Pharmacy, **24(7)**, 681-684, 1998.
8. Hammad, M.A., and Muller, B.W., Solubility and stability of clonazepam in mixed micelles, Int. J. Pharm., **169**, 55-64, 1998.
9. Martin, A., Physical chemical principals in the pharmaceutical sciences, Physical Pharmacy, Lea & Febiger, 371, 1993.
10. Shiau, B.J., Rouse, J.D., Sabatini, D.A., Harwell, J.H., Surfactant Selection for Optimizing Surfactant-Enhanced Subsurface Remediation, American Chemical Society, **6**, 65-79, 1995.
11. Lawrence, M.J., Surfactant Systems: Microemulsions and Vesicles as Vehicles for Drug Delivery. European Journal of Drug Metabolism and Pharmacokinetics, **3**, 257-269, 1994.
12. Evan, D.F., Wennerstrom, H., The colloidal domain where physics, chemistry, biology and technology meet, VCH Publisher, INC., 461-490, 1994.
13. Shinoda, K., Kunieda, H., Arai, T. and Saijo, H., Principles of Attaining Very Large Solubilization (Microemulsion): Inclusive Understanding of the Solubilization of Oil and Water in Aqueous and Hydrocarbon Media, J. Phys. Chem., **88**, 5126-5129, 1984.

14. Malcolmson, C. and Lawrence, M.J., Three-component non-ionic oil-in-water **4**, 97-109, microemulsions using polyoxyethylene ether surfactants, Colloids and Surface B: Biointerfaces, 1995.
15. Aboofazeli, R., Lawrence, C.B., Wicks, S.R., and Lawrence, M.J., Investigations into the formation and characterization of phospholipid microemulsions. III. Pseudoternary phase diagrams of systems containing water-lecithin- isopropyl myristate and either an alkanolic acid, amine, alkanediol, polyethylene glycol alkyl ether or alcohol as cosurfactant, Int. J. Pharm., **111**, 63-72, 1994.
16. Singh, H.N., Durge Prasad, C., Kumar, S., Water Solubilization in Microemulsions Containing Amines as Cosurfactant, JAOCS, **70(1)**, 69-73, 1993.
17. Schubert, K.V., and Kaler, E.W., Nonionic Microemulsions, Ber. Bubsenges, Phy. Chem., **3(100)**, 190-205, 1996.
18. Shinoda, K. and Lindman, B., Organized surfactant systems: microemulsions, Langmuir, **2(3)**, 135-149, 1987.
19. Aboofazeli, R., Patel, N., Thomas, M., and Lawrence, M.J., Investigations into the formation and characterization of phospholipid microemulsions. IV. Pseudoternary phase diagrams of systems containing water-lecithin-alcohol and oil; influence of oil, Int. J. Pharm., **125**, 107-116, 1995.
20. Thevenin, M.A., Grossiord, J.L. and Poelman, M.C., Sucrose ester/cosurfactant microemulsion systems for transdermal delivery: assessment of bicontinuous structures, Int. J. Pharm., **137**, 177-186, 1996.
21. Aboofazeli, R. and Lawrence, M.J., Investigations into formation and characterization of phospholipid microemulsions. I. Pseudoternary phase diagrams of systems containing water-lecithin-alcohol-isopropyl myristate, Int. J. Pharm., **93**, 161-175, 1993.
22. Kahlweit, M., Busse, G., and Faulhaber, B., Preparing Microemulsions with Lecithins, Langmuir, **11**, 1576-1583, 1995.
23. Hutchison, K., Digestible emulsions and microemulsions for optimum oral delivery of hydrophobic drugs, Gattefosse N 87, 67-74, 1994.
24. Gattefosse, Material safety data sheet and microemulsions for topical application, 10<sup>th</sup> pharmaceutical technology conference.
25. Attwood, D., Mallon, C., Ktistis, G., and Taylor, C.J., Study on the factors influencing the droplet size in nonionic oil-in-water microemulsions, **88**, 417-422, 1992.
26. Attwood D., Microemulsions, J. Kreuter (ed) Colloid Drug Delivery Systems, Marcel Dekker, New York, **66**, 1994.

27. Rosoff, M., and Serajuddin, A.T.M., Solubilization of diazepam in bile salts and sodium cholate-lecithin-water phases, Int. J. Pharm., **6**, 137-146, 1980.
28. Dunn, R.O., and Bagby, M.O., Solubilization of methanol and triglycerides: Unsaturated long-chain fatty alcohol/medium-chain alkanol mixed amphiphile systems, J. Am. Oil Chem. Soc., **71(1)**, 101-108, 1994.
29. Mitra, N., Mukherjee, L., Bhattacharya, P.K., and Moulik, S.P., Biological microemulsions V: Mutual mixing of oils, amphiphiles and water in ternary and quaternary combinations, Indian Journal of Biochemistry & Biophysics, **33**, 206-212, 1996.
30. Bhargava, H.N., Naruskar, A. and Lieb, L.M., Using microemulsions for drug delivery, Pharm Tech., **03**, 46-54, 1987.
31. Joubran, R.F., Cornell, D.G. and Parris, N., Microemulsions of triglyceride and non-ionic surfactant-effect of temperature and aqueous phase composition, Colloids and Surfaces A: Physicochemical and Engineering Aspect, **80**, 153-160, 1993.
32. Rosano, H.L., U.S. Patent 4, 27 March, 146-149, 1979.
33. Gradzielski, M., and Hoffmann, H., Charged O/W microemulsion droplets, Progress in Colloid & Polymer Science, **93**, 167-174, 1993.
34. Trevino, S.F., Joubran, R., Parris, N., and Berk, N.F., Structure of a triglyceride microemulsion: A small angle neutron scattering study, Langmuir, **10**, 2547-2552, 1994.
35. Kratochvil, J., Size of bile salt micelles: Techniques, Problems and Results, Advances, Colloid and Interface Science, **26**, 131-151, 1986.
36. Friberg, S.E., Micelles, microemulsions, liquid crystals, and the structure of stratum corneum lipids, J. Soc. Chem., **41**, 155-171, 1990.
37. Regev, O., Ezrahi, S., Aserin, A., Garti, N., Wachtel, E., Kaler, W., Khan, A., and Talmon, Y., A Study of the microstructure of a four-component nonionic microemulsion by Cryo-TEM, NMR, SAXS, and SANS, Langmuir, **12**, 668-674, 1996.
38. Gorski, N., Gradzielski, M., Hoffmann, H., The Influence of Ionic Charges on the Structural and Dynamic Behavior of O/W Microemulsion Droplets, Physical Chemistry, **100**, 1109-1117, 1996.
39. Fletcher, P.D.I., Morris, J.S., Turbidity of oil-in-water microemulsion droplets stabilized by nonionic surfactant, J. Colloids Surfaces A: Physicochemical and Engineering Aspects, **98**, 147-154, 1995.

40. Hunter, R.J., Foundations of Colloid Science, Vol. II. University of Sydney, Oxford University Press, Waton street, Oxford, 987-988, 1995.
41. Sjoblom, E. and Friberg, S., Light-Scattering and Electron Microscopy Determinations of Association Structures in w/o microemulsions, J. Colloid Interface Sci., **62(1)**, 16, 1978.
42. Green, J., Electron microscope study of a glass-forming water/oil pseudo- three-component microemulsion systems, J. Phy. Chem., 94, 1990.
43. Wadle, A., Forster, Th., and Rybinski, W.V., Influence of the microemulsion phase structure on the phase inversion temperature emulsification of polar oils, Colloids and Surfaces A: physicochemical and Engineering Aspects, **76**, 51-57, 1993.
44. Paul, C.H., Raj, R., Principles of Colloid and Surface Chemistry, 3rd edition, Marcel Dekker Inc., 236, 1990.
45. Bond, C., Clausse, M., Lagourette, B. and Peyrelasse, J., Detection of Structural transitions in Water-in-Oil Microemulsion-Type Systems through Conductivity and Permittivity Studies, J. Phys. Chem., **84**, 1520-1525, 1980.
46. Baker, R.C., Florence, A.T., Ottewill, R.H. and Tadros, Th.F., Investigations into the Formation and Characterization of Microemulsions II. Light Scattering Conductivity and Viscosity Studies of Microemulsions, J. of Colloid and interface Sci., **100(2)**, 332, 1984.
47. Zourab, S., Miller, C., Equilibrium and dynamic behavior for systems containing nonionic surfactants, n-hexadecane, triolein and oleyl alcohol, Colloids and Surfaces A: Physicochemical and Engineer Aspects, **95**, 173-183, 1995.
48. Lagues, M. and Sauterey C., Percolation transition in Water in Oil microemulsions. Electrical Conductivity Measurements, J. Phys.Chem., **84**, 3503-3508, 1980.
49. Kunieda, H., Nakano, A., and Angeles Pes, M., Effect of Oil on the Solubilization in Microemulsion Systems Including Nonionic Surfactant Mixtures, Langmuir, **11**, 3302-3306, 1995.
50. Alander, J. and Warenheim, T., Model Microemulsions Containing Vegetable Oils Part 1: Nonionic Surfactant Sytems, J. Am. Oil Chem. Soc., **66(11)**, 1656, 1989.
51. Croda, The Croda product guide to innovative pharmaceutical raw materials, Croda Inc., Parsippany, N.J, 1997.
52. Lobo, M., Master thesis., University of Rhode Island, 1998.

53. Merk Index of chemicals and drugs, 7th edition, Merck & Co Inc., Rahway, N.J, 1960.
54. Malcolmson, C., Satra, C., Kantaria, S., Sidhu, A., and Lawrence, M.J., Effect of oil on the level of solubilization of testosterone propionate into nonionic oil-in-water microemulsions, J. Pharm. Sci., **87**, 109-116, 1998.
55. Nash, J.F., Gettings, S.D., Diembecks, M., and Chudowski, M., A Toxicological Review of Topical Exposure to White Mineral Oils, Ed Chem, **34(2)**, 213-225, 1996.
56. Shinoda, K. and Lindman, B., Organized Surfactant Systems: Microemulsions, Langmuir, **3(2)**, 135-148, 1987.
57. Friberg, S., Lapezynska, I. and Gillberg, G., Microemulsions Containing Nonionic Surfactants - The Importance of the PIT Value, J. Colloid Interface Sci., **56**, 19, 1976.
58. Rosen, M.J., Gemini surfactants, Cosmet. Toiletries, **113**, 49-55, 1998.
59. Kon-no, K. and Kitahara, J. Colloid Interface Sci., **34**, 221, 1970.
60. Schott, H. and Royce, E., Effect of Inorganic Additives on Solutions of Nonionic Surfactants VI: Further Cloud Point Relations, J. of Pharm. Sci., 73(6), 793, 1984.
61. Kahweit, M., Strey, R., Firman, P., Haase, G., Jen, J. and Schomacker, R., General Patterns of the Phase Behavior of Mixtures of H<sub>2</sub>O, Nonpolar Solvent, Amphiphiles, and Electrolytes, Langmuir **4**, 499-511, 1988.
62. Wiencek, J.M. and Qutubuddin, S., Solubilization in nonionic microemulsions, Colloids and Surfaces, 54, 1-23, 1991.
63. Gan Zou, L., Gui Ying, X., Hong Zui, M.A.O., Li Qiang, Z., and Hong Li, W., Formation and Characteristics of Middle-phase Microemulsions-Effect of Synergism of Surfactant, Alcohol, and Oil, Chi. Sci. Bulletin, **39(4)**, 292-296, 1994.
64. De Vos, A., Vervoort, L., and Kinget, R., Solubilization and stability of indomethacin in a transparent oil-water gel, Int. J. Pharm, **92**, 191-196, 1993.
65. Trotta, M., Pattarino, F., and Gasco, M.R., Influence of counter ions on the skin permeation of methotrexate from water-oil microemulsions, Pharmaceutica Acta Helvetiae, **71(1)**, 135-140, 1996.
66. Jayakrishnan, A., Kalaiarasi, K., and Shah D.O., Microemulsions: Evolving technology for cosmetic applications, J. Soc. Cosmet. Chem., **34**, 335-350, 1983.



67. Asano, H., Sasamoto, H., and Ueno, M., Solubilization of cholesterol in two binary mixed micelles of bile salt and nonionic surfactant, J. Am. Oil Chem. Soc., **71**(1), 1994.
68. Khairi, M.S., Timoney, R.F., and Corrigan, O.I., Micellar solubilization of clofazimine analogues in aqueous solutions of ionic and nonionic surfactants, Pharmaceutical Research, **10**( 4), 631-634, 1993.
69. Teelmann.K., Schlappi, B., Schupbach, M., and Kistler, A., Preclinical Safety Evaluation of Intravenously Administered Mixed Micelles, Biological Pharmaceutical Research Department. Hoffmann-La Roche & Co., Switzerland, 1517-1523, 1984.
70. Fahelbom, K.M.S., Timoney, R.F., and Corrigan, O.I., Micellar solubilization of clofazimine analogues in aqueous solutions of ionic and nonionic surfactants, Pharmaceutical Research, **10**( 4), 631-634, 1993.
71. Wilkins, J.B., Moore, R.J., 7th Ed. Chemical Publishing, New York, Harry's Cosmeticology, 1982.
72. Fox, C., Cosmetic and pharmaceutical vehicles: antiperspirants, cleansers and emulsions, Cosmetics & Toiletries, **112**(12), 31-44, 1997.
73. Fox, C., Skin and skin care, Cosmetics & Toiletries, **113**, 21-24, 1998.
74. Gattefosse, Microemulsion formulation guide, Pharmaceutical department, edition 05/92.
75. Gulik-Krzywicki, T., and Larsson, K., An electron microscopy study of the L2-phase (microemulsion) in a ternary system: Triglyceride/monoglyceride/water, Chem. Phys. Lipids, **35**, 127, 1984.
76. Experimental Determination of "Required HLB" sheet, Henkel Corporation, Hoboken, N.J.
77. Wade A., and Weller J. P., Handbook of pharmaceutical excipients, 2 nd edition, edited by. The pharmaceutical Press, London, **316**, 1994.
78. Cruickshank, C.N.D., and Squire, J.R., Skin cancer in the engineering industry from the use of mineral oil. Brishtish Journal of Medicine, **50**, 289, 1993.
79. William. D.F., and Schmitt, W.11., Chemistry and Technology of The cosmetics and Toiletries industry, 20.1992.
80. Croda analysis report of super refined soybean oil USP/NP, manufacture date 9/28/99, test date 9/28/99, Croda Inc.
81. Myddleton, W.W., Chemistry of oils, fats , and waxes, Chapter VI, 61-77.

82. Certified of analysis sigma aldrich product sheet, <https://www.sigma-aldrich.com/sacatalog>, 83.

## 7. BIBLIOGRAPHY

Aboofazeli, R., Lawrence, C.B., Wicks, S.R., and Lawrence, M.J., Investigations into the formation and characterization of phospholipid microemulsions. III. Pseudoternary phase diagrams of systems containing water-lecithin- isopropyl myristate and either an alkanolic acid, amine, alkanediol, polyethylene glycol alkyl ether or alcohol as cosurfactant, International Journal of Pharmaceutics, **111**, 63-72, 1994.

Aboofazeli, R. and Lawrence, M.J., Investigations into formation and characterization of phospholipid microemulsions. I. Pseudoternary phase diagrams of systems containing water-lecithin-alcohol-isopropyl myristate, International Journal of Pharmaceutics, **93**, 161-175, 1993.

Aboofazeli, R., Patel, N., Thomas, M., and Lawrence, M.J., Investigations into the formation and characterization of phospholipid microemulsions. IV. Pseudoternary phase diagrams of systems containing water-lecithin-alcohol and oil; influence of oil, International Journal of Pharmaceutics, **125**, 107-116, 1995.

Alander, J. and Warenheim, T., Model Microemulsions Containing Vegetable Oils Part 1: Nonionic Surfactant Systems, Journal of American Oil Chemistry Society, **66(11)**, 1656, 1989.

Asano, H., Sasamoto, H., and Ueno, M., Solubilization of cholesterol in two binary mixed micelles of bile salt and nonionic surfactant, Journal of American Oil Chemistry Society, **7(1)**, 1994.

Attwood, D., Mallon, C., Ktistis, G., and Taylor, C.J., Study on the factors influencing the droplet size in nonionic oil-in-water microemulsions, **88**, 417-422, 1992.

Attwood D., Microemulsions in J. Kreuter (ed) Colloid Drug Delivery Systems, Marcel Dekker, New York, **66**, 1994.

Baker, R.C., Florence, A.T., Ottewill, R.H. and Tadros, Th.F., Investigations into the Formation and Characterization of Microemulsions II. Light Scattering Conductivity and Viscosity Studies of Microemulsions, Journal of Colloid and Interface Science, **100(2)**, 332, 1984.

Bhargava, H.N., Naruskar, A. and Lieb, L.M., Using microemulsions for drug delivery, Pharmaceutical Technology, **03**, 46-54, 1987.

Bond, C., Clause, M., Lagourette, B. and Peyrelasse, J., Detection of Structural transitions in Water-in-Oil Microemulsion-Type Systems through Conductivity and Permittivity Studies, Journal of Physical Chemistry, **84**, 1520-1525, 1980.

Certified of analysis sigma aldrich product sheet, <https://www.sigma-aldrich.com/sacatalog>.

Croda analysis report of super refined soybean oil USP/NP, manufacture date 9/28/99, test date 9/28/99, Croda Inc.

Constantinides, P., Lancaster, C.M., Marcello, J., Chiossone, D.C., Orner, D., Hidalgo, I., Smith, P., Sarkahian, A., Yiv, S.H., and Owen, A., Enhanced intestinal absorption of an RGD peptide from water-in-oil microemulsions of different composition and particle size, Journal of Controlled Release, **34**, 109-116, 1995.

Croda, The Croda product guide to innovative pharmaceutical raw materials, Croda Inc., Parsippany, N.J, 1997.

Cruickshank, C.N.D., and Squire, J.R., Skin cancer in the engineering industry from the use of mineral oil, British Journal of Medicine, **50**, 289, 1993.

Dangi, J.S., Vyas, S.P., and Dixit, V.K., The role of mixed micelles in drug delivery. I. Solubilization. Drug Development and Industrial Pharmacy, **24(7)**, 681-684, 1998.

De Vos, A., Wervoort, L., and Kinget, R., Solubilization and stability of indomethacin in a transparent oil-water gel, International Journal of Pharmaceutics, **92**, 191-196, 1993.

Dunn, R.O., and Bagby, M.O., Solubilization of methanol and triglycerides: Unsaturated long-chain fatty alcohol/medium-chain alkanol mixed amphiphile systems, Journal of American Oil Chemistry Society, **71(1)**, 101-108, 1994.

Evan, D.F., Wennerstrom, H., The colloidal domain where physics, chemistry, biology and technology meet, VCH Publisher, INC., 461-490, 1994.

Fletcher, P.D.I., Morris, J.S., Turbidity of oil-in-water microemulsion droplets stabilized by nonionic surfactant, Journal of Colloids Surfaces A: Physicochemical and Engineering Aspects, **98**, 147-154, 1995.

Experimental Determination of "Required HLB" sheet, Henkel Corporation, Hoboken, N.J.

Fahelbom, K.M.S., Timoney, R.F., and Corrigan, O.I., Micellar solubilization of clofazimine analogues in aqueous solutions of ionic and nonionic surfactants, Pharmaceutical Research, **10( 4)**, 631-634, 1993.

Fox, C., Cosmetic and pharmaceutical vehicles: antiperspirants, cleansers and emulsions, Cosmetics & Toiletries, **112(12)**, 31-44, 1997.

Fox, C., Skin and skin care, Cosmetics & Toiletries, **113**, 21-24, 1998.

Fox, C., Cosmetic Emulsions In: Emulsion and Emulsion Technology Part II. Surfactant Science Series, Marcel Dekker Press, New York, **6**, 1974.

Friberg, S.E., Micelles, microemulsions, liquid crystals, and the structure of stratum corneum lipids, Journal of Society Chemistry, **41**, 155-171, 1990.

Friberg, S., Lapezynska, I. and Gillberg, G., Microemulsions Containing Nonionic Surfactants - The Importance of the PIT Value, Journal of Colloid and Interface Science, **56**, 19, 1976.

Gan Zou, L., Gui Ying, X., Hong Zui, M.A.O., Li Qiang, Z., and Hong Li, W., Formation and Characteristics of Middle-phase Microemulsions-Effect of Synergism of Surfactant, Alcohol, and Oil, Chinese Science Bulletin, **39(4)**, 292-296, 1994.

Gattefosse, Microemulsion formulation guide, Pharmaceutical department, edition 05/92.

Garcia-Celma, M.J., Solubilization of Drugs in Microemulsions in Industrial Applications of Microemulsions., Marcel Dekker, New york.

Gulik-Krzywicki, T., and Larsson, K., An electron microscopy study of the L2-phase (microemulsion) in a ternary system: Triglyceride/monoglyceride/water, Lipids, **35**, 127, 1984.

Gorski, N., Gradzielski, M., Hoffmann, H., The Influence of Ionic Charges on the Structural and Dynamic Behavior of O/W Microemulsion Droplets, Physical Chemistry, **100**, 1109-1117, 1996.

Gradzielski, M., and Hoffmann, H., Charged O/W microemulsion droplets, Progress in Colloid & Polymer Science, **93**, 167-174, 1993.

Green, J., Electron microscope study of a glass-forming water/oil pseudo- three-component microemulsion systems, Journal of Physical Chemistry, **94**, 1990.

Hammad, M.A., and Muller, B.W., Solubility and stability of clonazepam in mixed micelles, International Journal of Pharmaceutics, **169**, 55-64, 1998.

Hunter, R.J., Foundations of Colloid Science Vol. II. University of Sydney, Oxford University Press, Waton street, Oxford. 987-988, 1995.

Hutchison, K., Digestible emulsions and microemulsions for optimum oral delivery of hydrophobic drugs, Gattefosse N 87, 67-74, 1994.

Jayakrishnan, A., Kalaiarasi, K., and Shah D.O., Microemulsions: Evolving technology for cosmetic applications, Journal of Society of Cosmetic Chemistry, **34**, 335-350, 1983.

Joubran, R.F., Cornell, D.G. and Parris, N., Microemulsions of triglyceride and non-ionic surfactant-effect of temperature and aqueous phase composition, Colloids and Surfaces A: Physicochemical and Engineering Aspect, **80**, 153-160, 1993.

Kahlweit, M., Busse, G., and Faulhaber, B., Preparing Microemulsions with Lecithins, Langmuir, **11**, 1576-1583, 1995.

Kahlweit, M., Strey, R., Firman, P., Haase, G., Jen, J. and Schomacker, R., General Patterns of the Phase Behavior of Mixtures of H<sub>2</sub>O, Nonpolar Solvent, Amphiphiles, and Electrolytes, Langmuir, **4**, 499-511, 1988.

Kahlweit, M., Strey, R. and Husse, G., Phase Behavior of Multicomponent Systems Water-Oil-amphiphile-Electrolyte. 3, Journal of Physical Chemistry, **89**, 163-171, 1995

Khairi, M.S., Timoney, R.F., and Corrigan, O.I., Micellar solubilization of clofazimine analogues in aqueous solutions of ionic and nonionic surfactants, Pharmaceutical Research, **10**( 4), 631-634, 1993.

Kon-no, K. and Kitahara, Journal of Colloid and Interface Science, **34**, 221, 1970.

Kratohvil, J., Size of bile salt micelles: Techniques, Problems and Results, Advances in Colloid and Interface Science, **26**, 131-151, 1986.

Kunieda, H., Nakano, A., and Angeles Pes, M., Effect of Oil on the Solubilization in Microemulsion Systems Including Nonionic Surfactant Mixtures, Langmuir, **11**, 3302-3306, 1995.

Lagues, M. and Sauterey C., Percolation transition in Water in Oil microemulsions. Electrical Conductivity Measurements, Journal of Physical Chemistry, **84**, 3503-3508, 1980.

Lawrence, M.J., Surfactant Systems: Microemulsions and Vesicles as Vehicles for Drug Delivery, European Journal of Drug Metabolism and Pharmacokinetics, **3**, 257-269, 1994.

Lobo, M., Master thesis., University of Rhode Island, 1998.

Malcolmson, C., Satra, C., Kantaria, S., Sidhu, A., and Lawrence, M.J., Effect of oil on the level of solubilization of testosterone propionate into nonionic oil-in-water microemulsions, Journal of Pharm. Science, **87**, 109-116. 1998.

Malcolmson, C. and Lawrence, M.J., Three-component non-ionic oil-in-water **4**, 97-109, microemulsions using polyoxyethylene ether surfactants, Colloids and Surface B:Biointerfaces, 1995.

Martin, A., Physical chemical principals in the pharmaceutical sciences, Physical Pharmacy, Lea & Febiger, 371, 1993.

Merk Index of chemicals and drugs, 7th edition, Merck & Co Inc., Rahway, N.J, 1960.

- Myddleton, W.W., Chemistry of oils, fats, and waxes, Chapter VI, 61-77.
- Mitra, N., Mukherjee, L., Bhattacharya, P.K., and Moulik, S.P., Biological microemulsions V: Mutual mixing of oils, amphiphiles and water in ternary and quaternary combinations. Indian Journal of Biochemistry & Biophysics, **33**, 206-212, 1996.
- Nash, J.F., Gettings, S.D., Diembecks, M., and Chudowski, M., A Toxicological Review of Topical Exposure to White Mineral Oils, Faraday Chemistry, **34(2)**, 213-225, 1996.
- Paul, C.H., Raj, R., Principles of Colloid and Surface Chemistry, 3rd edition, Marcel Dekker Inc., 236, 1990.
- Regev, O., Ezrahi, S., Aserin, A., Garti, N., Wachtel, E., Kaler, W., Khan, A., and Talmon, Y., A Study of the microstructure of a four-component nonionic microemulsion by Cryo-TEM, NMR, SAXS, and SANS, Langmuir, **12**, 668-674, 1996.
- Rosano, H.L., U.S. Patent 4, 27 March. 146-149, 1979.
- Rosano, H., Cavallo, J.L., Chang, D., and Whittam, J.H., Microemulsions: A commentary on their preparation. Journal of Society Chemistry, **39**, 201-209, 1988.
- Rosoff, M., and Serajuddin, A.T.M., Solubilization of diazepam in bile salts and sodium cholate-lecithin-water phases, International Journal of Pharmaceutics, **6**, 137-146, 1980.
- Rosen, M.J., Gemini surfactants. Cosmetic Toiletries, **113**, 49-55, 1998.
- Schott, H. and Royce, E., Effect of Inorganic Additives on Solutions of Nonionic Surfactants VI: Further Cloud Point Relations, Journal of Pharmaceutical Sciences, **73(6)**, 793, 1984.
- Schubert, K.V., and Kaler, E.W., Nonionic Microemulsions. Ber. Bubsenges, Physical Chemistry, **3(100)**, 190-205, 1996.
- Sjoblom, E. and Friberg, S., Light-Scattering and Electron Microscopy Determinations of Association Structures in w/o microemulsions, Journal of Colloid and Interface Science, **62(1)**, 16, 1978.
- Shiau, B.J., Rouse, J.D., Sabatini, D.A., Harwell, J.H., Surfactant Selection for Optimizing Surfactant-Enhanced Subsurface Remediation, American Chemical Society, **6**, 65-79, 1995.
- Shinoda, K., Kunieda, H., Arai, T. and Saijo, H., Principles of Attaining Very Large Solubilization (Microemulsion): Inclusive Understanding of the Solubilization of Oil and Water in Aqueous and Hydrocarbon Media, Journal of Physical Chemistry, **88**, 5126-5129, 1984.

Shinoda, K. and Lindman, B., Organized surfactant systems: microemulsions, Langmuir, **2(3)**, 135-149, 1987.

Singh, H.N., Durge Prasad. Ch., Kumar, S., Water Solubilization in Microemulsions Containing Amines as Cosurfactant, Journal of American Oil Chemistry Society, **70(1)**, 69-73, 1993.

Swarbrick, J., and Boylan, J., Microemulsions: Encyclopedia of pharmaceutical Technology, **9**, 392-396, 1988.

Teelman, K., Schlappi, B., Schupbach, M., and Kistler, A., Preclinical Safety Evaluation of Intravenously Administered Mixed Micelles, Biological Pharmaceutical Research Department, Hoffmann-La Roche & Co., Switzerland, 1517-1523, 1984.

Thevenin, M.A., Grossiord, J.L. and Poelman, M.C., Sucrose ester/cosurfactant microemulsion systems for transdermal delivery: assessment of bicontinuous structures, International Journal of Pharmaceutics, **137**, 177-186, 1996.

Trevino, S.F., Joubran, R., Parris, N., and Berk, N.F., Structure of a triglyceride microemulsion: A small angle neutron scattering study, Langmuir, **10**, 2547-2552, 1994.

Trotta, M., Pattarino, F., and Gasco, M.R., Influence of counter ions on the skin permeation of methotrexate from water-oil microemulsions, Pharmaceutica Acta Helveticae, **71(1)**, 135-140, 1996.

Usacheva, T.M., Lifanova, N.V., and Zhuralev, V.I., Dielectric Properties of Nonionic Microemulsions, Colloid Journal, **59**, 79-85, 1997.

Wade A., and Welier J. P., Handbook of pharmaceutical excipients, 2 nd edition, edited by, The pharmaceutical Press, London, **316**, 1994.

Wadle, A., Forster, Th., and Rybinski, W.V., Influence of the microemulsion phase structure on the phase inversion temperature emulsification of polar oils, Colloids and Surfaces A: physicochemical and Engineering Aspects, **76**, 51-57, 1993.

Wienczek, J.M. and Qutubuddin, S., Solubilization in nonionic microemulsions. Colloids and Surfaces, **54**, 1-23, 1991.

Wilkinson, J.B., Moore, R.J., 7th Ed. Chemical Publishing, New York, Harry's Cosmeticology, 1982.

William, D.F., and Schmitt, W.H., Chemistry and Technology of The cosmetics and Toiletries industry, 20, 1992.



Zourab, S., Miller, C., Equilibrium and dynamic behavior for systems containing nonionic surfactants, n-hexadecane, triolein and oleyl alcohol. Colloids and Surfaces A: Physicochemical and Engineer Aspects. **95**, 173-183, 1995.